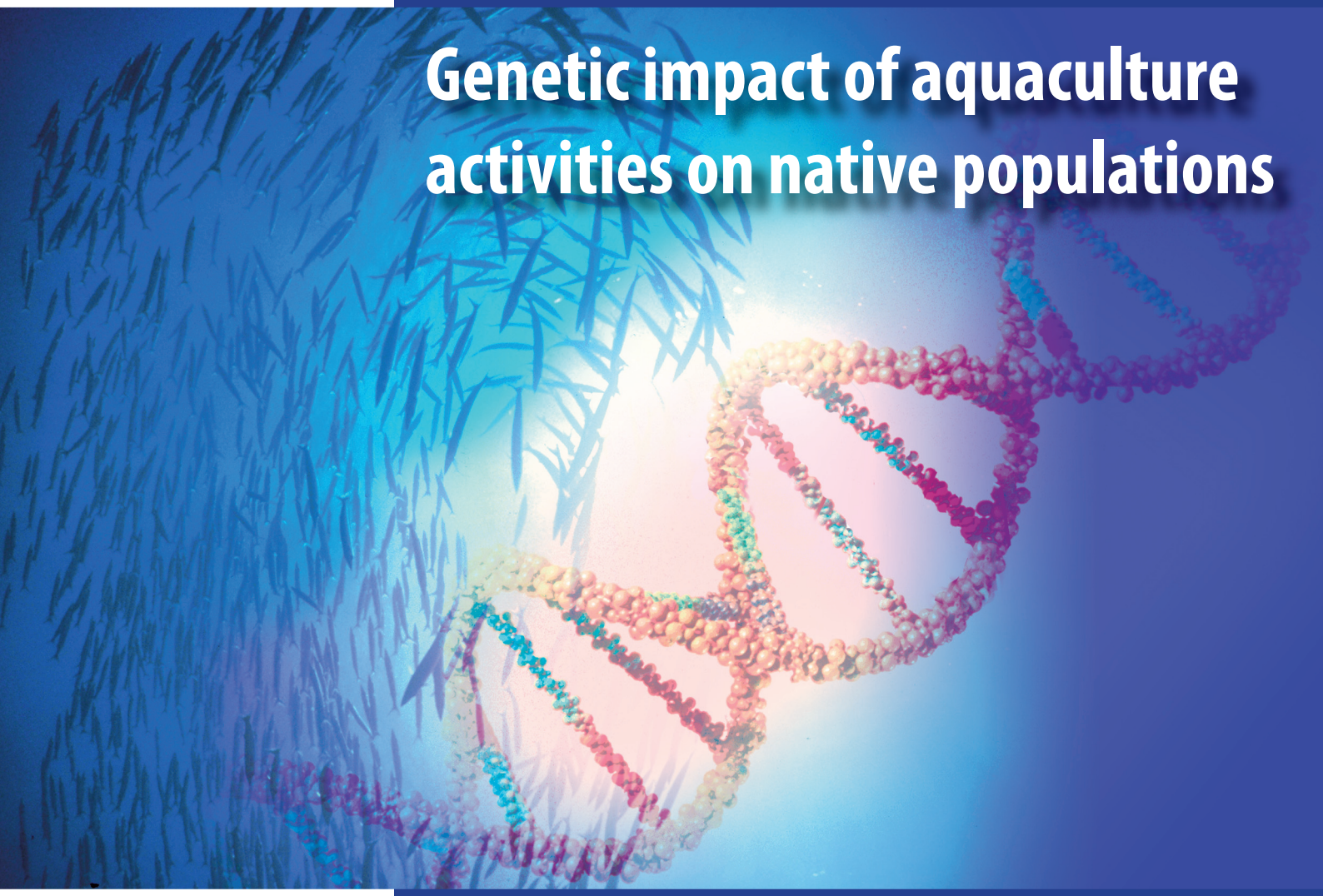
GENIMPACT

Genetic impact of aquaculture activities on native populations



Terje Svåsand, Donatella Crosetti, Eva García-Vázquez, Eric Verspoor (lead editors)

6th Framework plan
of the European Commission

**Genimpact- Evaluation of genetic impact of aquaculture activities on native populations.
A European network (EU contract n. RICA-CT-2005-022802). Final scientific report, July 2007**

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The Genimpact project

The project Genimpact, financed by the European Commission, started in November 2005 to review existing knowledge necessary to assess genetic effects of aquaculture on biodiversity, review future research needs, and disseminate this information to a wider public. To achieve this, Genimpact convened a series of expert workshops on risk assessment and interbreeding and aquaculture-ecosystem interactions:

I *Genetics of domestication, breeding and enhancement of performance of fish and shellfish*. Viterbo, Italy, 12 -17 June 2006

II *Monitoring tools for evaluation of genetic impact of aquaculture activities on wild populations*. Tenerife, Spain, 19 - 21 October 2006

III *The use of modelling to assess the risk of genetic impacts on wild populations from escapes of cultured fish*.
Pitlochry, Scotland, UK, 15 - 17 February 2007

IV *Development of management options to reduce genetic impacts of aquaculture activities*. Thessaloniki, Greece, 19 - 22 April 2007.

This publication presents the outputs of these four workshops.

Each section is composed of a series of chapters, and while the report represents an integrated whole, each chapter can stand alone as an independent document with regards to some particular subtopic. Some additional linking paragraphs are added at the beginning of each section to insure that the chapters are appropriately put in context.

Index

I

I

Genetics of domestication, breeding and enhancement of performance of fish and shellfish

I.1 *Genetic effects of domestication, culture and breeding of fish and shellfish, and their impacts on wild populations*

These chapters report an updated overview of the knowledge available on the genetic effects of domestication, culture and breeding of fish and shellfish, and their impacts on wild populations, for the 12 species/groups of species considered in Genimpact, and recommend specific research priorities for the future.

Participants to the Viterbo (Italy) workshop, 12nd-17th June 2006

Front row (from left): *F. Piferrer, A.R. Rossi, L. Colombo, D. Danancher, E. Garcia Vazquez, S. Lapègue, G. Hulata, J. McElwee, T. Svåsand, L.Sola*

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Atlantic cod - *Gadus morhua*

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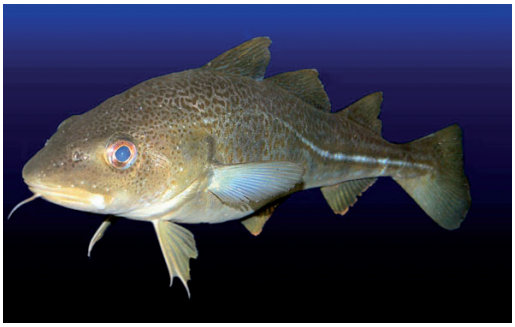

Biology, ecology and genetics

Distribution and capture

Cod inhabit the continental shelves and banks in most areas in the North Atlantic (1) and are therefore distributed in many different environments with respect to temperature and salinity (Fig. 1). A comprehensive overview of biological characters for each population can be found in International Council for Exploration of Sea (2).

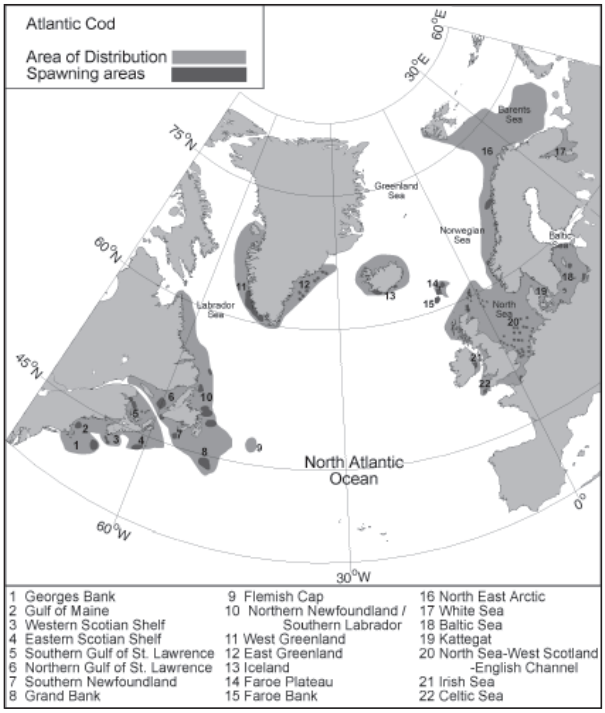

Fig. 1. *Distribution and spawning areas of all major cod stocks.*

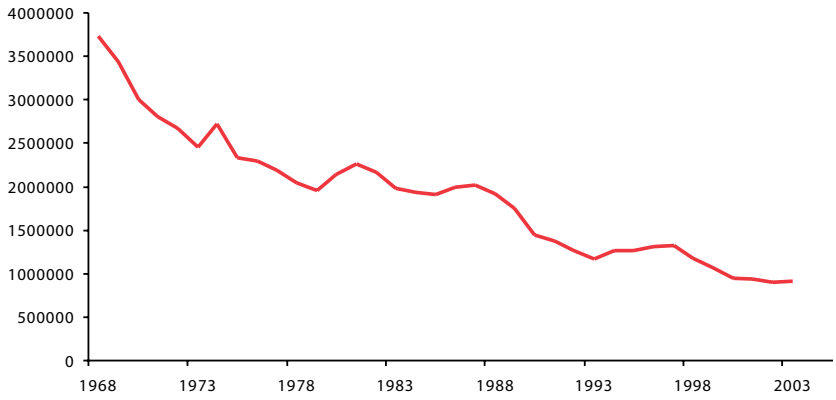

Fig. 2. *Total catch (tonnes) of cod in the N. Atlantic (3,4).*

Capture

Abundance of cod varies greatly among the different areas of the North Atlantic. Most of the cod stocks have been exposed to high fishing mortality. In 1970, the total catch was about 3.5 million tonnes in North Atlantic (Fig. 2). Today the total catch has declined to less than a million tonnes. As a result, most of the cod stocks have declined and many have collapsed (3).

Biology

The cod has a long historical record as an important marine resource throughout the distribution range. It is a bottom fish species mainly distributed from the shoreline and down to the continental shelf. The spawning normally occurs in winter / spring on well known spawning sites (2). Mature cod migrate to the spawning sites where they aggregate and spawn. It is a very fecund species with numbers of eggs ranging from thousands to 20-30 millions per female (5). During each season the females spawn several batches of eggs, which are fertilised externally. The eggs are pelagic, and egg buoyancy and the hydrographical conditions are important factors for determining the geographical distribution (4, 6). The cod larvae are also pelagic and they first feed on plankton (copepod larvae), then krill and other small crustaceans. At larger size cod mainly feed on fish. During summer or fall the fry change their pelagic distribution and settle to the bottom.

There are large variation between different populations with regard to larval distribution and migration back to spawning sites. Most extreme is the Northeast Arctic cod population, which has the main spawning sites located near the Lofoten Island on the Norwegian coast. The eggs and larvae are drifting north with the coastal current and are distributed over a large area in the Barents Sea - Spitsbergen. In connection with spawning, the cod has to migrate from the Barents Sea and back to the spawning sites around Lofoten, which involve large distances (2).

Population genetics

The relationship between the stationary and migratory cod populations was discussed in details more than 100 years ago (7). First genetic studies were started during the 1960s. Haemoglobin was the first genetic marker that was used to study cod populations (8), and large differences in allele frequencies were found in more detailed studies (9, 10). The haemoglobin results and other studies on blood proteins (11) supported the view that cod could be divided into migratory arctic cod, and more stationary coastal cod. However, the variation in allele frequency of haemoglobin along the Norwegian coast could also be explained by selection due to different environmental conditions (10). Studies employing allozyme markers have shown relatively limited variation among cod populations along the Norwegian coast (12, 13).

More recent studies employing various DNA markers have yielded results ranging from panmixia or high gene flow (mtDNA) across much of the Atlantic (14, 15) to the presence of significant population structuring (microsatellite DNA, scnDNA) on small to medium spatial scales (16, 17, 18). Similar results have been reported from the western Atlantic, where temporally stable differences between inshore and offshore cod off Newfoundland have been demonstrated (19). The gene marker Pan I exhibits particularly large differences in allele frequencies between samples collected in the Barents Sea and in coastal areas of Norway. While samples of Northeast Arctic cod are almost fixed for the Pan IB allele, samples of coastal cod exhibit high frequencies of the Pan IA allele (17, 18).

The large genetic variation between cod from different areas, even in minor geographic scale, suggests that many cod populations are adapted to local environmental conditions (20).

Breeding and culture practices

Production

Atlantic cod farming is predicted to become the second most economically important marine finfish species, after Atlantic salmon, to be farmed in Europe. Present global production figures are shown in Tab.1 (21) and the preliminary results for 2005 are 7 000 tonnes. This is relatively low. However, confident industry analysis predicts annual harvests of 175 000 tonnes by 2010 (22).

	2000	2001	2002	2003	2004
Norway	169	864	1,253	2,185	3,168
Iceland	<0.5	140	192	380	636
UK		15			8
Total	*169.5*	*1,019*	*1,445*	*2,565*	*3,812*

Tab. 1. *Atlantic cod aquaculture production (tonnes)*

Hatchery practices

Broodstock for farming come from different geographically areas and most hatcheries in Norway have mixtures of north east arctic cod and coastal cod. Cod easily spawn in captivity, and due to the high fecundity, large amounts of high quality eggs and larvae can be produced. When cod are held in intensive culture conditions they mature within 2 years from hatching (23), much earlier than their wild counterparts (24). Such "early" maturation has significant impacts on potential production profitability as maturation reduces growth potential. Thus, it extends the production cycle (by a minimum of 6 months) and has negative impacts on the product quality. Cod eggs are usually incubated at 5-7° C and hatch after 15-20 days. The larvae start feeding 4-5 days after hatching.

Extensive and semi-extensive methods for larvae production rely on natural plankton for start feeding. Most hatcheries today have an intensive production which includes live food production, feeding larvae with rotifers and artemia (25).

Grow-out

There are two main methods of cod farming in Iceland and Norway: one is capturing and on-growing of wild cod, the other is production of cod from hatching to market size.

The number of escaped cod in Norway for 2003 and 2004 was 75 000 individuals and the estimate for 2005 is approx 167 000 individuals. It is discussed whether cod itself is able to make holes in the seine as behaviour of cod is different from that of e.g. salmon. The Norwegian Research Council is financing a project called "Why and how does cod escape?", which includes behaviour studies both under experimental and commercial farming conditions. The goal is to use this information to suggest methods for surveillance, recapt©ure of escaped fish and to predict

possible interactions. Norway has a law regulating all aspects of aquaculture, and escapes from farming have to be reported to the Directory of Fisheries immediately. In serious cases it can also be reported to the police.

Selective breeding

As intensive farming of cod has had renewed interest the last years, selective breeding programmes have been initiated both in Norway and Iceland. The Norwegian Institute of Fisheries and Aquaculture Research has the responsibility for the national breeding programme financed by the Ministry of Fisheries and Coastal affairs. MarineBreed, a commercial company, has also started a breeding programme. In Iceland the breeding programme for cod has resulted in a company (Icecod) owned by both governmental institutes and private companies (26). Canada started in 2005 a programme called "Atlantic Cod Genomics and Broodstock Development" (27). The aim of the programme is to develop tools to identify cod with superior traits of commercial importance. Base populations in the Norwegian programmes have been produced from both North East Arctic cod and coastal populations. Testing and breeding strategies follows very much breeding programmes for Atlantic salmon. The three European breeding programmes have carried out one round of selection based primarily on growth rate but also to some extent included disease resistance, early maturation and quality traits. A combined family and individual selection is used. The test fish in the breeding programmes are farmed in different environments (geographically) and the results will provide an indication of possible genotype-environment interactions.

Until recently very few quantitative genetic studies were carried out in cod, but from recent years some results have been published. So far the results show heritability at the same magnitude as for many other fish species (28-31).

Tests for parental assignment in cod have been developed based on microsatellite markers (32) and the breeding programme in Iceland is using genotyping instead of separate rearing of families. Studies on possible QTLs for disease resistance and quality traits are ongoing and could be included in breeding programmes by Marker-Assisted-Selection.

Interaction studies

The development of cod aquaculture has raised questions about possible environmental effects of cod farming, including possible genetic influence of farmed populations on wild populations. Farmed cod could be equipped with a genetic marker that would positively identify the cod as a farmed one. This gives a unique possibility for tracing the effects of escape and in-cage spawning on the wild populations. The genetic marker for cod was developed at the institute of Marine Research in the 1980s (33), and a new cod broodstock with the same gene marker is now under development.

Much research is going on to prevent early maturation. This is important both for aquaculture and for possible gene interactions between farmed and wild fish. The Institute of Marine Science in Norway started 2006 a study about the effects of spawning in net pens.

Prevention of escape must be achieved through technical changes adapted surveillance and increased knowledge about cod's behaviour in cages. The Directorate of Fisheries, Norway, has initiated in the spring 2006 a plan to reduce escapees from aquaculture. One out of 29 proposed actions is the consideration of using sterile fish in aquaculture.

Conclusions/Implications

Cod is one of the most important marine resources in the north Atlantic and consists of a number of populations, in some cases even within a minor geographic range. Several of these are depleted, and with respect to small populations such as the Norwegian coastal cod, these are possibly more vulnerable to human impacts including aquaculture.

The interest in cod farming is increasing in several countries, and similar environmental problems are expected as have been experienced in the salmon farming industry. Commercial cod farming is developing rapidly in several countries, and cod breeding programmes have already been initiated in Norway and Iceland.

Although studies on the possible gene interactions between wild and farmed or ranched cod have already started (33, 34), as much knowledge as possible is required to be prepared for the expected increase in cod farming. More information about the small local populations such as coastal cod in Norway is required, especially to detect local adaptations (35). In this regard it is urgent to conduct "common garden" experiments to evaluate population specific life history characteristics which are relevant for local adaptation.

Research to reduce escapees must have high priority. Since cod also spawn in net pens the possibility of sterile fish will be investigated. The goal for a breeding programme (and farming/management in general) should be cod reaching harvest size before maturation. Studies on genetic variation for age at sexual maturation should be prioritised to include this in the breeding criteria (in addition to other ways of reducing the problem of early maturation).

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Atlantic halibut - *Hippoglossus hippoglossus*

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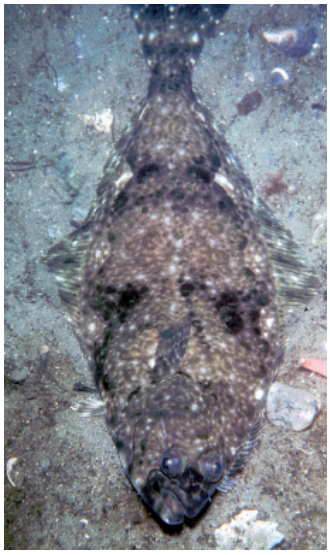

Biology, ecology and genetics

Distribution

The Atlantic halibut is distributed throughout the northern part of the Atlantic Ocean and in parts of the Arctic Ocean (Fig.1) (1). The species is particularly abundant along the Norwegian coast, the Faroe Islands, Iceland, and southern Greenland. It may also be encountered in the North Sea and the western part of the Baltic Sea. Along the east coast of North America, the halibut is distributed from Hudson Strait southward to the southern Grand Bank and St. Pierre Bank.

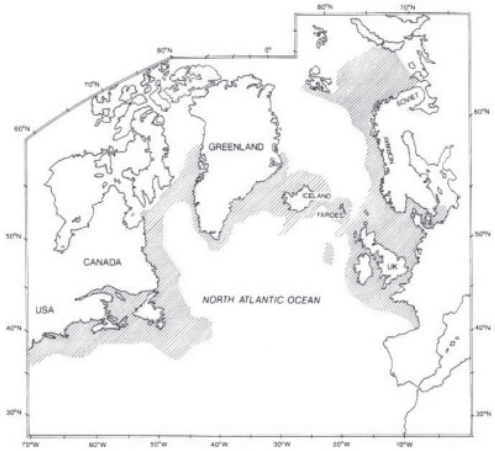

Fig. 1. *Distribution of Atlantic halibut (hatched areas) in the North Atlantic (1)*

Capture

The Atlantic halibut has been an attractive species for European fishermen, and high fishing intensity has resulted in depletion of stocks in several areas (2). The capture of Atlantic halibut in Europe has declined from an all time high of 10 000-15 000 tonnes in the period 1950 to 1965, to less than 2 000 tonnes in 2004 (3). In 2004, the main countries fishing for Atlantic halibut were Norway (1,034 tonnes), Iceland (574 tonnes), Faeroe Islands (497 tonnes), and United Kingdom (251 tonnes).

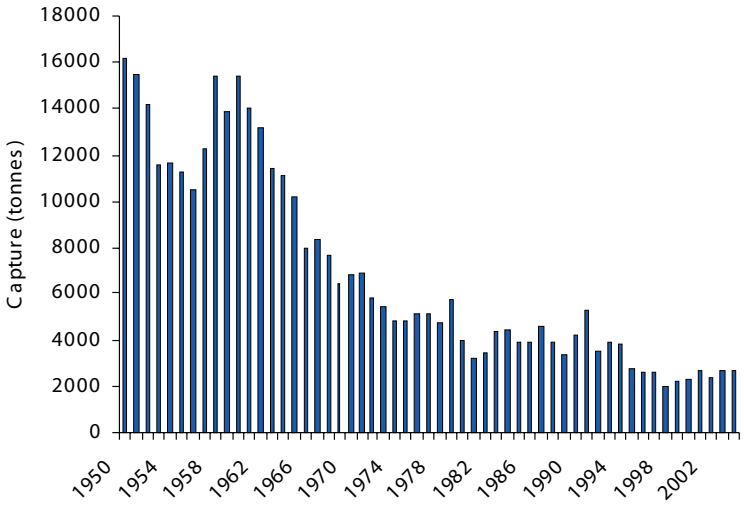

Fig. 2. *Capture of Atlantic halibut in Europe (3)*

Biology

The Atlantic halibut is a long-living flat fish that may exceed weights in excess of 250kg. Spawning occurs in deep water (300-700m) in the period December to March, although peak activity is generally observed in January and February. The early life-history stages of the Atlantic halibut in the wild are poorly understood, however, it is known that both the planktonic eggs and larvae may drift significant distances with ocean currents. After metamorphosis juvenile halibut adopt a benthic life-style. Coastal areas with depths of 20-60m often serve as nursery areas before the halibut undertake migrations to distant areas, both shallow and deep waters. The species displays sex-dependant growth rates where males attain sexual maturity at a younger age and smaller size than females. In a study of maturation age in the Faroe Islands in the 1980s, the average age of sexual maturation in males was estimated at 4.5 years, 55cm and 1.7kg, whilst in females it was 7 years, 110-115cm and 18kg (2). However, age at first maturity displays individual, temporal and spatial variation. After spawning halibut leave the spawning grounds and may be found in deep and shallow waters, both inshore and offshore. The Atlantic halibut is a batch spawner and may release eggs every 3rd day for up to 15 batches in any given spawning season.

Population genetics

Early tagging experiments demonstrated that mature Atlantic halibut return to the same spawning site over repeated spawning seasons (2). Such observations lead to the suggestion that the species may display population genetic structuring (4). Evidence from biochemical genetic studies has revealed either a lack of or only weak evidence of population genetic differentiation between geographically distinct samples (5, 6, 7, 8). A more recent study utilising highly polymorphic microsatellite DNA markers revealed a lack of population genetic structuring (9). However, this study utilised small sample sizes and considered only a limited geographic area. It is possible that as is the situation for other marine species, a greater number of polymorphic markers and more intense sampling may illustrate population genetic structuring in this species. Until such a large-scale study has been conducted, it is not possible to draw any conclusions regarding the population genetic structure of this species.

Certain traits that may be under quantitative genetic control (e.g., growth, timing of spawning) show phenotypic variation among halibut collected from geographically distinct locations. For example, earlier spawning is generally recorded in Atlantic halibut in northern Norway compared to southern Norway. However, the biological significance of such variation, and the relative importance of genetics and environment on these differences are not known. Consequently, it is at present unclear as to whether or not the Atlantic halibut displays local adaptation or not.

Breeding and culture practices

Production

Experimental aquaculture of Atlantic halibut was initiated in Norway and Iceland in the mid 1980s, however, it was not before the late 1990s that significant commercial production was realised.

	1998	1999	2000	2001	2002	2003	2004	2005 (provisional)
Norway			100	100	100	500	500	500
Iceland		453	5	189	250	250	250	300
UK	20	50	30	100		95	105	105
Total	*20*	*503*	*135*	*389*	*350*	*845*	*855*	*905*

Tab. 1. *Halibut aquaculture production (in tonnes) (10)*

Whilst yearly production fluctuated in the period 1998-2005, there has been a general increase, and at present approximately 1 000 tonnes is produced by aquaculture on a yearly basis within Europe. Norway is the primary producer of Atlantic halibut followed by UK and Iceland. It is likely that within the next decade, aquaculture production of Atlantic halibut will overtake wild capture.

Due to its good taste and limited availability, Atlantic halibut is often regarded as an exclusive product with prices that reflect this. Whole fish can reach market prices of 10-15 €, making it an economically attractive species for further aquaculture development.

Hatchery practices

Broodfish used for gamete collection are most often wild caught individuals that are kept in captivity. Whilst some high performing F1 individuals are also used for gamete collection, a practise that varies greatly from farm to farm, and country to country, the majority of juvenile production still arises from wild broodfish.

Ripe females kept in large shallow tanks are coaxed onto stripping boards that are often neoprene covered to protect the slime layer. Eggs are stripped into jars and fertilised externally with sperm from 1-3 stripped males. The process from incubation to weaning is best carried out at approx. 6°C although temperatures between 3-8°C are tolerated for this phase. Eggs are incubated in the dark in cylinders where they are suspended in the column through continuous flow of cooled and preferably often sterile salt water. After approximately 2 weeks (82 degree-days), eggs hatch and the resultant yolk-sac larvae are transferred to larger incubation silos which may vary between 1 and 15 cubic meters. Start-feeding has historically been the major bottleneck in halibut juvenile production where in excess of 75% mortality at this stage was common in early attempts. Both semi-intensive and intensive feeding methods have been utilised although intensive methods are almost exclusively used at present. Light quality and strength was demonstrated to be of significance in addition to quality of Artemia to feed upon. Addition of algae and zooplankton to the tanks has also increased survival during this critical phase. After metamorphosis where the larvae become fry and the left eye migrates to the right eye, the halibut will often seek the bottom of the tank. At this stage they can be reared in very

shallow flat-bottomed tanks that are either round or long with water inlet at the top end. They are gradually weaned onto commercial feeds and reared in this manner until approximately 5g.

Grow-out

From 5g to approximately 30-50g the halibut continue to be reared in shallow land-based through-flow tanks fed with commercial diet. At this stage halibut can be transferred to adapted marine cages which have flat bottoms with tight mesh so that they can rest on them. Some cover may be required for these relatively shallow cages in order to avoid fish from becoming sun burnt. Size at slaughter is preferably over 5kg although 3-5kg fish are also marketable. Grow-out usually takes 2-4 years of cage rearing.

Selective breeding

The life-cycle for Atlantic halibut was first closed in culture in Norway in 1992/93 when offspring from the 1985 year class were first used for breeding at the Institute of Marine Research Austevoll research station outside Bergen. However, despite the life-cycle having been closed since the early 1990´s, at present the majority of halibut production is still based upon wild captured individuals being used as broodstock. No systematic selective breeding programme for Atlantic halibut is in operation in any country in Europe, although this topic has been addressed on several occasions, most recently in a Norwegian meeting run by the Norwegian Flatfish Forum (March 2006).

In a recent experimental breeding project funded by the Norwegian Research Council, 22 family groups were produced in 1997-1998 (11). In this study, family survival rate varied from 50 to 84,6% and between 30 and 78,8% for the 1997 and 1998 year classes respectively. Mean weight varied from 1.0 to 1,6 kg at ca 3½ years for the 1997 year class and from 0.4 to 1.1 at 2½ years age for the 1998 year class. The significant differences in both growth and survival suggest additive genetic variation for these traits. This has the potential for exploitation in a future commercial breeding programme. Although a breeding plan has been designed for Atlantic halibut in Norway (12), there is first a need for more knowledge about the spawning process of halibut in order to obtain a more stable egg quality and control maternal effects.

A small and initial breeding programme has been conducted on the West Atlantic (Canada). An attempt to establish a low cost breeding programme for halibut in Scotland without rearing families separately until tagging failed due to too big variation in survival of different family groups, with a consequently low effective population size and increased inbreeding rate (13).

Concentration of research on other production-limiting factors such as weaning, diet and pigmentation problems have received a greater focus than establishment of halibut breeding. However, it is likely that a selective breeding programme will be initiated in the near future.

Interaction studies

At present, there are no available data on the number of farmed escaped halibut in the wild. However, due to the fact that many halibut are reared in land-based facilities where escapement to the wild is either difficult or impossible, and the level of production is still low, it is likely that there are few farmed escapees. No scientific

studies investigating the numbers of escapees, and genetic or ecological interactions of potential escapees in native stocks have been conducted. Furthermore, no studies have been carried out looking into the potential genetic or ecological differences between cultured and wild Atlantic halibut.

Due to the fact that aquaculture production of this species is currently based upon collecting gametes from wild captured adults that are not the product of a selective breeding programme, it is likely that the genetic differences between the F1 farm progeny and wild individuals is limited. However, it is predicted that the genetic difference between wild and cultured Atlantic halibut will increase if a selective breeding programme for this species is successfully implemented.

Conclusions/Implications

Throughout most of its range, the Atlantic halibut has already been heavily exploited through over-fishing, and it is likely that this leaves the species in a vulnerable state with respect to potential impacts from escapees from halibut culture or further exploitation. At the present level of aquaculture production however, and with the use of wild caught broodstock for gamete collection, it is likely that genetic impacts of escapees on wild populations are minimal. However, this topic has not been studied, and this situation may quickly change if a selective breeding programme for this species is initiated and the level of production increases.

Clearly, in order to be able to predict or understand the potential genetic interactions between farmed and wild Atlantic halibut, a significantly better understanding of its population genetic structure and potential fitness-related genetic differences this may give rise to, is required. Furthermore, a better understanding of the biology and ecology of the halibut in the wild, especially with respect to the early life-history stages, is required.

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Atlantic salmon – *Salmo salar*

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Biology, ecology and genetics

Distribution

The species' range encompasses Europe, North America, and Greenland (1) (Fig. 1). Non-anadromous forms occur in Europe in a few land-locked locations in Norway and Sweden, and throughout the Neva/Lake Ladoga system and Karelia regions of Russia. In North America non-anadromous forms occurred throughout most of the species' historical range, as far west as Lake Ontario, and remain common throughout Newfoundland, Labrador and northern Quebec. Over the last 150 years anadromous stocks have become extinct in many rivers. Native stocks are no longer present in the Elbe and the Rhine, two of Europe's largest rivers, or in many rivers draining into the Baltic and in southern England, France and Spain, which previously had abundant salmon runs (1), and have become extinct in many rivers in the US and southern Canada (1, 2, 3). The species has been introduced in a number of locations, both within its range and elsewhere (1) but its native range has contracted and fragmented. Most remaining stocks, particularly in the southern part of its range and in the Baltic Sea region, are depressed and many no longer self-sustaining; detailed information, however, is lacking for most river systems where the species still occurs.

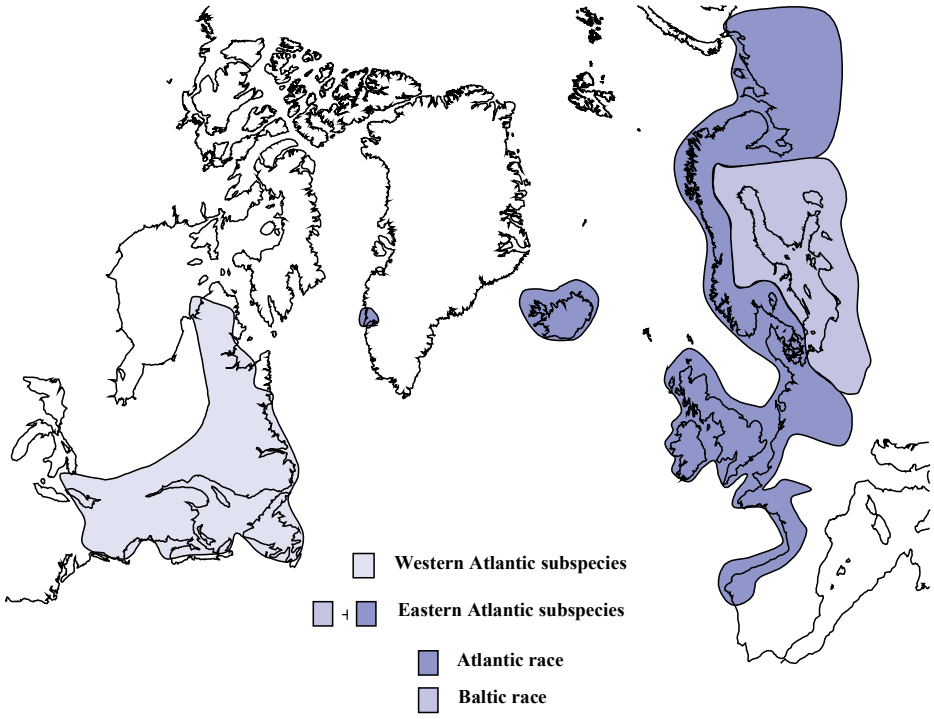

Fig. 1. *The historical distribution of Atlantic salmon and its main evolutionary divisions.*

Biology

Atlantic salmon, *Salmo salar,* are salmonid fishes of the temperate and subarctic regions of the North Atlantic (4). They show strong homing to natal areas to spawn and are highly variable in their biology within and among locations. Fertilization is external with eggs generally deposited in gravel nests in rivers and covered by the female. Over 70% of natural mortality occurs from the egg to fry stage. Juveniles feed on small invertebrates and adults on crustaceans and fish. Most stocks are anadromous - egg and juvenile development occur in freshwater, as smolts they migrate to the sea, where they feed, mature, and they return to rivers to spawn and complete their life-cycle (Fig.2); migrations in rivers may exceed 1 000 km and in the sea 2 000 km. Some salmon are non-anadromous, completing their entire life cycle in freshwater (Fig.2), and migrate from spawning rivers to lakes, or are locally resident in either rivers or lakes. Anadromous forms spend 1-7 years in freshwater and 1-4 years at sea; variable proportions of male parr mature in fresh water and contribute to spawning, and some spawners survive, migrate back to sea, and return to spawn again. Adults range from ~50-120 cm and ~2-30 kg; females produce ~2 000 – 15 000 eggs. Their marine phase is poorly understood as is, to a lesser degree, the ecology of eggs and alevins in river gravels (4). Resident fish mature at age 2-5, can live for 10 or more years, growing to ~12-120 cm and ~20 g to 20 kg, depending on location, with spawning in consecutive years common. Small females produce as few as 33 eggs and some populations are lake spawning.

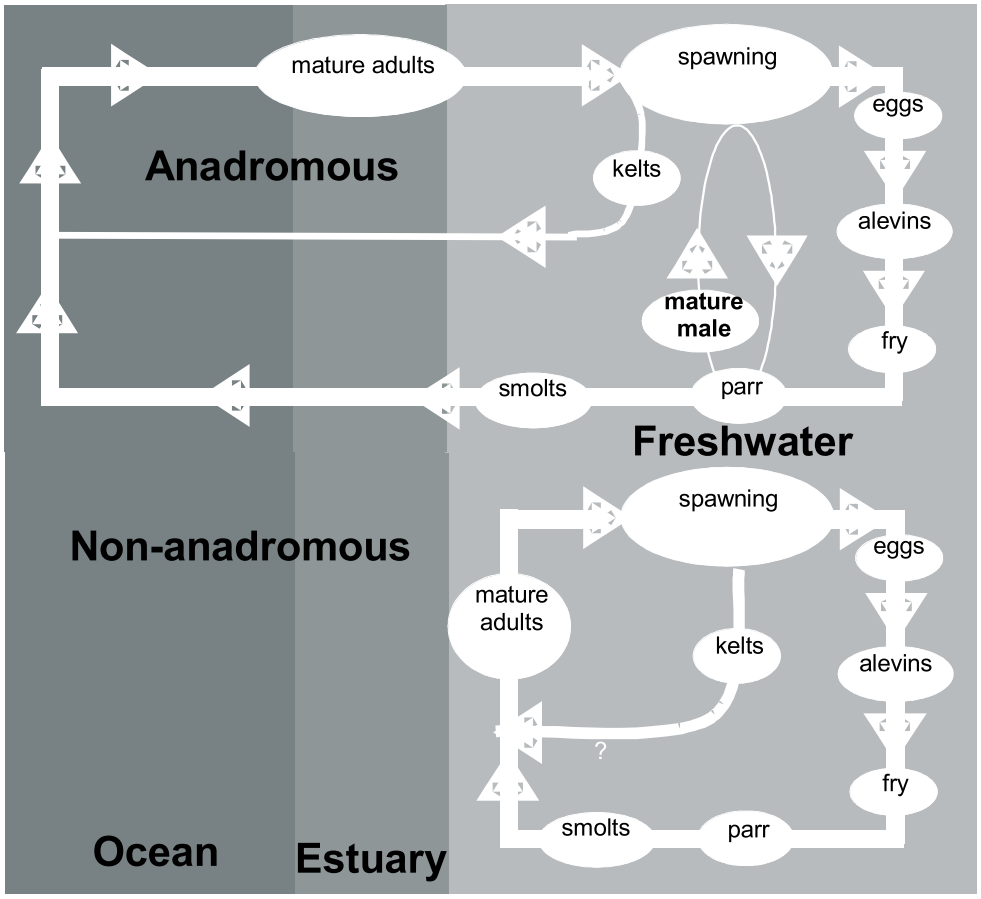

Fig. 2. *The basic life cycles of anadromous and non-anadromous Atlantic salmon*

Capture

Exploitation in the North Atlantic, in decline over the last half century (5) (Fig.3), encompasses high seas, coastal, river mouth fisheries, and in-river recreational fishing, with the latter making up to 80% of regional catches and 17-87% of fish caught in rivers released after capture. In the Baltic Sea coastal and river fisheries have become relatively more important than sea fisheries, but overall fisheries catches have declined and only ~1 000 tonnes of wild fish were caught in 2004(6).

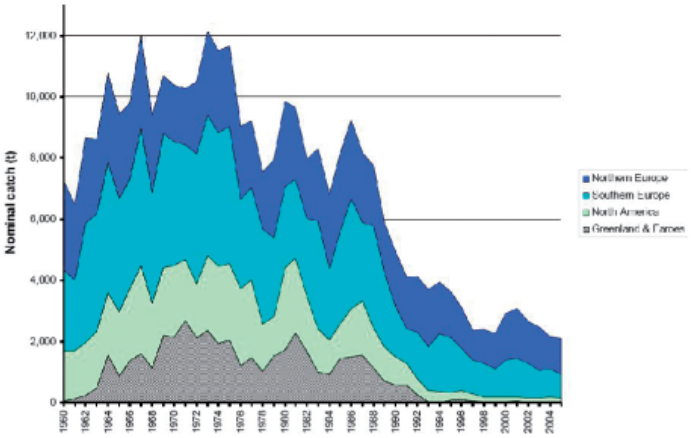

Fig. 3. *The nominal North Atlantic catch of Atlantic salmon since 1960 - excludes Baltic Sea (5).*

Population genetics and local adaptation

Conditions necessary for population structuring exist: homing to natal rivers to spawn, a naturally fragmented distribution of spawning and juvenile habitat, and a capacity for local adaptation. The existence of structuring and highly restricted contemporary gene flow, even among tributaries within many rivers, is indicated by observed molecular genetic differentiation (7). Limited but sporadic gene flow among populations may locally link populations within or among rivers into evolutionarily connected meta-population groups, but this is poorly understood. Genetic isolation has been sufficient for phylogenetic and evolutionary divergence at all spatial scales (7) and divides the species into two distinct subspecies (Fig. 3), *Salmo salar salar* Linnaeus 1758 and *S. salar sebago* Girard 1854, respectively, which have been largely isolated for >500 000 yrs (7); these encompass, respectively, salmon in the eastern Atlantic and the other salmon in the western Atlantic. The former are further divided into two less divergent, more recently evolved "races", one associated with Atlantic and the other with Baltic drainages (Fig. 3). Further regional evolutionary divisions occur within these three groups, and non-anadromous stocks have evolved independently in most river systems (7).

Stocking of fish has been carried out across the species European range, to supplement depressed river stocks, e.g. where spawning grounds have been lost through hydroelectric developments, and to restore extinct stocks, but is in decline and increasingly tightly controlled. Increased abundance is seen in some cases but most attempts to restore self-sustaining salmon populations have failed and its value appears variable and is in many situations questionable at best.

Conditions for local adaptation of Atlantic salmon exist and the evidence it occurs is compelling, though largely circumstantial (8). High heritability values are seen

for variation in fitness-related traits such as growth and body composition, and to a lesser extent for health traits such as disease resistance, and for survival and life history traits. Also, genotype-environment interactions and genetic correlates occur for many traits, translocations of salmon generally fail, performance of domesticated stocks in the wild is poor, performance differences occur among wild stocks in common-garden experiments, and there are non-random patterns of inherited resistance to some parasites in the wild. Through the process of domestication cultured stocks become adapted to a culture environment. A major component of local adaptation is likely to involve a genetic response to water quality, photoperiod and related variables, and disease vectors, as they are of particular biological importance and can vary spatially in a predictable way, likely to promote adaptive evolutionary change. However, local adaptation most likely varies spatially and can be expected to be lower within meta-populations.

Breeding and culture practices

Production

European farming started in 1969 and was >800 000 tonnes in 2004 (5) (Fig.4), ~60% of world and > 90% of North Atlantic production, is valued at US$ 141 billions (9) and is 300-600 times the nominal catch of wild salmon. Norway is Europe's biggest producer (565,902 tonnes) followed by the UK (158,099 tonnes) and the Faeroe Islands (37,296 tonnes). Ranched production in the Baltic involved the release of 5.28 million in 2004 (4.6-6.5 million/yr from 1987-2004) and contributed ~50% of the Baltic fisheries catch (6), though data show significantly lower survival to fishery for ranched smolts. Elsewhere in Europe, only ~10 tonnes of ranched salmon are now harvested (5).

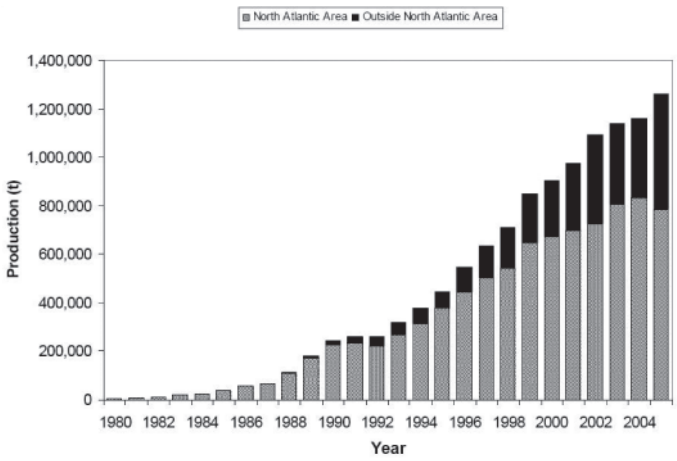

Fig. 4. *Farm production of Atlantic salmon since 1980 (5).*

Hatchery practices

Broodstock are normally moved from sea cages into freshwater tanks in autumn prior to stripping, producing ~1,600 eggs per kg body weight (10). Eggs are stripped, fertilized with milt, hardened in water, and disinfected prior to placing in hatchery trays or silo systems for incubation. Following eyeing, they are "shocked" to remove unfertilized and dead eggs. Hatching occurs in trays or, following transfer, in tanks. Both eggs and alevins are normally reared in water at <9 °C.

Grow out

Late alevins are transferred into tanks with flow-through or recirculation systems, and fed on inert feeds until smolts; this takes 1½ years on ambient temperature and light regimes, but as is now normal, light and temperature regimes are manipulated to reduce this to 6-8 months; in some areas parr are transferred for on-growing to cages in lakes. Production densities may be up to 50 kg/m^3 or higher (10). When smolts, at 40-120 g, fish are transferred to marine net cages anchored to the seabed, where temperatures are 6-16 °C and salinities near oceanic levels (33-34‰), with stocking densities of up to 20 kg/m^3; cages of 40 m in diameter and 20-30 m depth, and ten or more grouped together at a site, with most sites having only a single generation of fish. Rearing in pump-ashore seawater tanks is the exception. On-growing in cages lasts 1-2 years before harvesting at 2+ kg by netting and live transfer to slaughter plants by well-boats. Location of farms is regulated to ensure current speeds are sufficient to eliminate waste and supply well oxygenated water (~ 8 ppm), and takes proximity to other farms and/or wild fisheries into account. Sites are generally fallowed for 6 or more weeks before a new generation of fish is introduced.

Selective breeding

The European industry is now largely based on a few selectively bred strains of mostly Norwegian origin. The first, established in the early 1970s (10) with 190 males and 430 females from 40 Norwegian river, encompassed four distinct strains to deal with the species' 4-year generation interval in culture. There are now four breeding programmes in Norway providing ~80% of Norwegian farms with smolts. Two utilise combined family and within-family selection programmes and each comprises a breeding station, several commercial farms and fish labs, and commercial multiplier hatcheries to produce smolts for farms. Trait selection started with growth and now encompasses sexual maturation, disease resistance, and carcass quality. Such broad breeding goals result in a low selection pressure across a wide spectrum of alleles. Programmes involve testing 300-400 families per year with effective breeding populations of 33-125 individuals, sufficient for short-term inbreeding avoidance. Little genetic exchange occurs between Norwegian programmes but fish are widely exported within and outside Europe. Similar family based breeding programmes exist in Iceland, Ireland, Faeroe Islands and Scotland.

Differentiation from wild stocks

Genetically differentiation from wild populations is expected due to: 1) the effects of limited numbers, non-random selection and sourcing of wild founders, 2) domestication selection, 3) loss of variability by genetic drift (increased by using small numbers of brood fish), and 4) selective breeding for economic traits (11). Differences have been reported with regard to variation at protein genes, and at mitochondrial and nuclear DNA loci as well as for phenotype variation (6). Molecular studies show reductions in numbers of alleles and mean heterozygosity of up to 50% and differentiation between strains and wild founder populations 2-6 times higher than among wild populations in general. Changes due to domestication and trait selection exist for growth rate, body size, survival, delayed maturity, stress tolerance, temperature tolerance, disease resistance, flesh quality and egg production. Unintentional correlated changes also occur for fitness-related traits including survival, deformity, spawning time, morphology, fecundity and egg viability, aggression, risk-taking behaviour, and growth hormone production. A >100% and 20% genetic gains have been recorded for growth and feed conversion

efficiency, respectively after 5-6 generations in one Norwegian farm strain (12); not surprisingly, farm salmon outgrow wild salmon both in culture and the wild (11).

No commercial production of triploid or transgenic salmon occurs in Europe. The former produce sterile fish but there is concern over the use due to reduced growth rate and deformities, and marketability is hindered by consumer perception that they are GMO´s. Salmon transgenic for a growth hormone gene have been engineered and are awaiting US FDA approval, and a transgenic freeze-resistant salmon is under development for aquaculture in low temperatures.

Interaction studies

Around 0.5-2 million salmon (0.5-1.6% of production) escape each year into the North Atlantic, ~50% of the wild pre-fishery abundance in the region (9), despite close regulation of farming. Numbers of sexually mature farm salmon returning to rivers in the 1980s and 1990s ranged from 200 000-300 000 and composed up to 80% of salmon in some Norwegian rivers (9). Escapes generally enter rivers, near to their farm of origin, but some may do so hundreds of kilometres away, where many interbreed with wild fish, with farm females generally more successful than farm males and more hybrid than pure offspring produced. Escapes of juveniles from hatcheries and freshwater cages occur in some areas but their numbers and impact are poorly documented.

Both can cause direct genetic effects on wild populations (9).

Predictions of impact based on modelling vary, depending on the assumptions made. In field studies indirect effects are seen due to behavioural, ecological, and disease interactions which can reduce effective sizes of wild populations and increase genetic drift; in particular, competition with farm fish and hybrids, which are larger, may depress wild smolt production (11). Direct effects occur due to interbreeding. Farm and farm-wild hybrid offspring show substantially reduced lifetime success with poorer survival in freshwater and at sea, causing reduced recruitment of wild fish (11). When farm escapes are re-occurring, fitness reductions may accumulate and can lead to the extinction of already depressed populations. Change may also occur in the character of wild populations. For example, increases may occur in multi-sea-winter salmon in predominantly grilse populations. However, though more fecund, given their reduced lifetime success, such hybrids do not compensate for the loss of wild recruitment, and still decrease overall population fitness. Such negative consequences may not occur in all situations, but are likely to be widespread. Impacts are expected even if all wild populations were genetically identical as domestication appears to be the main reason for the genetic differences between farm and wild populations.

Conclusions/Implications

Conclusions

- The basic biology and distribution of the Atlantic salmon are well understood.
- Two distinct forms occur – anadromous and non-anadromous, both highly but variably, structured into populations and meta-populations with little gene flow.
- Evolutionary structuring, due to historical isolation, is seen at all spatial scales, including within rivers, and between defines Eastern and Western Atlantic stocks as distinct subspecies.

- Population and meta-population structuring, and the spatial boundaries of these genetic groups, within and among river systems, remains to be resolved in detail.
- Compelling, but largely circumstantial evidence, indicate genetic populations and meta-populations are generally locally adapted, though its degree and spatial scale remain unclear.
- Maintaining evolved adaptive population and meta-population structuring, and historical habitat conditions, is essential for healthy, self-sustaining wild stocks.
- Production of farm salmon is well documented and 300-600 times wild production.
- The biology of farm stocks is well understood, rearing practises well defined, and breeding programmes and culture highly standardised and carried out at a sophisticated level, and farming occurs in a well defined regulatory environment in Europe.
- Production is largely based on a few breeding strains largely established from wild Norwegian populations and developed using family based selection programmes with large effective population sizes, but showing genetic shifts due to founder effects; there is no commercial farm production of triploids or GMO salmon in Europe.
- Selective breeding for economic traits has focused on growth, disease resistance, delayed sexual maturity, and carcass quality.
- Farm salmon are genetically different from wild fish with respect to a range of molecular and phenotypic traits, and display reduced genetic variation though the majority of additive genetic variation for phenotypic traits has been retained.
- ~0.5-1.6% of farm salmon (0.5-2 million) escape, equal to ~50% of pre-fisheries abundance of wild fish, of which ~10% enter rivers where many interbreed with wild salmon and trout.
- Farm escapes can have significant direct and indirect negative impacts on wild populations by reducing productivity, though the range of scenarios producing significant impacts is unclear; interbreeding can directly impact by reducing mean fitness and indirect effects occur through competitive, disease and parasite interactions; studies show farm fish in the wild have severely reduced life-time fitness compared to wild fish with intermediate hybrid fitness.
- Modelling suggests the impact will depend on the magnitude and frequency of escapes, the "health" of the wild populations and the specific genetic differences between the farm and wild stocks involved, and that in the extreme may lead to extinction of wild populations.
- Effective containment and considered location of farms, involving epidemiological zones and vaccination programmes to control disease and parasites, as well as maximising domestication of farm strains, is the best ways to ensure avoidance of direct and indirect genetic impacts.
- The ability to identify farm fish has significant potential to help understand the extent of fish escapes, their causes, and their impacts.

Research priorities

- Detailing the evolutionary diversity and the spatial boundaries of genetic populations, the extent to which they are dynamically linked into meta-populations and are conditioned by historical and contemporary factors environmental influences, including understanding of the extent of genetically effective straying among populations through juvenile and adult dispersal.
- Developing roboust, cost-effective methods for monitoring diversity, abundance, and effective breeding size (N_e) of local populations and metapopulations, taking into account overlapping generations and mature male parr; obtaining historical data to assess change.
- Understanding the degree and scale of local adaptations, and selective forces and how these vary spatially, by measuring the heritability of fitness related traits in the wild and carrying out, common garden/reciprocal transfer experiments.
- Increasing knowledge of the marine ecology of anadromous populations and metapopulations, and how this varies, as increased marine mortality, underlies recent declines, in abundance.
- Resolving the genetic basis of local adaptation and domestication, and how the former can vary, to understand the basis of outbreeding depression when farm and wild stocks mix.
- Developing low-cost genetic and non-genetic methods for ID of farm fish.
- Building realistic working simulation models to assess impact risk across interaction scenarios, define research priorities, and assess the efficacy of different management strategies.
- Field research to "proof" simulation models, including studies of indirect genetic and ecological impacts from disease introduction and of density dependent population dynamics.

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Common carp - *Cyprinus carpio*

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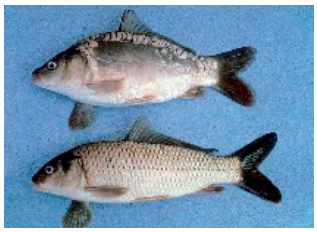

Biology, ecology and genetics

Distribution

The common carp [*Cyprinus carpio* Linnaeus (1758)] has been one of the oldest domesticated species of fish for food. Culture of carp in China dates back to at least the 5th century BC, although domestication began much later. The European races of carp derived from wild carp of the Danube; the earliest attempts date back to the Roman Empire and spread of Christianity in Europe, from where its domesticated forms were later introduced to other continents (1 and references therein, Fig.1). The common carp is divided into two subspecies, *C. c. carpio* from Europe and *C. c. haematopterus* from Asia, as reviewed by population genetic data (2 and references therein); populations of the Asian subspecies may be further subdivided into Central Asian and East/Southeast Asian ones (3). Productive populations were domesticated from both ancestral forms, as well as from their mutual hybrids and backcrosses followed by mass selection (2).

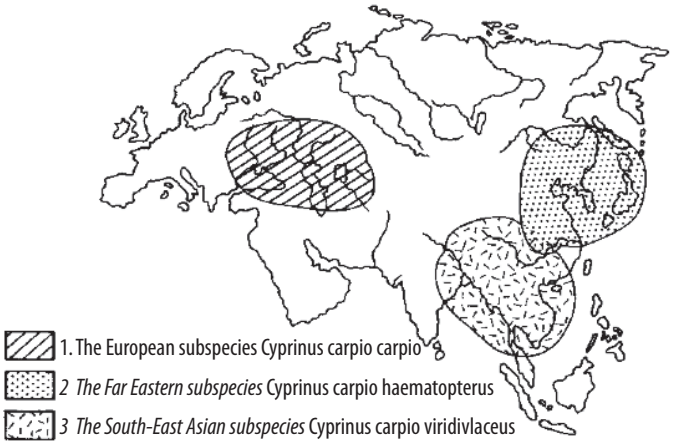

Fig. 1. *Ranges of wild common carp populations in Eurasia (4)*

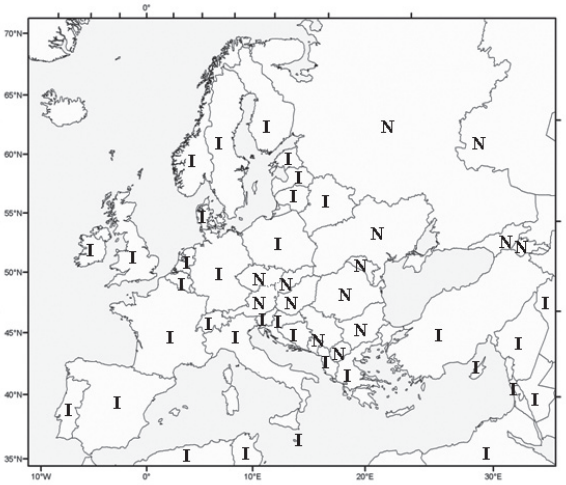

Fig. 2. *Status of common carp in Europe (N=native; I=introduced).*

Common carp is native to only a limited number of European countries, namely those of the Danube River drainage system. However, present occurrence of wild Danubian carp populations is questionable, probably limited to only a few areas in the drainage system, and are threatened by anthropogenic effects as well as farm escapees and restocking farmed populations into open waters (Fig.2). A few wild populations have recently been reported from Turkey, and although these are not native, they constitute an important resource. Wild stocks are also to be found in Central Asian countries, e.g. Uzbekistan, which cluster with the European populations.

Biology

Common carp dwells in middle and lower reaches of rivers and shallow confined waters. Best growth is obtained at water temperature of 23-30°C. The fish can survive cold winter periods. Salinity up to about 5‰ is tolerated, optimal pH is 6.5-9.0; common carp can survive low oxygen concentration (0.3-0.5 $mg.l^{-1}$) as well as supersaturation.

Carp are omnivorous, with a high tendency towards the consumption of benthic organisms, such as water insects, larvae of insects, worms, molluscs, and zooplankton. Digging in the bottom in search for food items results in turbid water. Zooplankton consumption is dominant in fish ponds where the stocking density is high. Additionally, the carp consumes the stalks, leaves and seeds of aquatic and terrestrial plants, decayed aquatic plants, etc. Typical carp ponds in Europe are shallow, eutrophic with a muddy bottom and dense aquatic vegetation at the dikes. Pond farming of common carp is based on natural food with supplemental feeding of cereals. Daily growth can be 2 to 4% of body weight (bw). Carps can reach 0.6 to 1.0 kg bw within one season in subtropical/tropical polyculture. Growth in temperate climate is slower, the fish reach 1.5 kg bw after 3 rearing seasons.

In Europe, females mature after 11 000 - 12 000 degree-days in the temperate and subtropical climatic zones; males mature 25-35% earlier. Maturity period of Asian breeds is slightly shorter (5). The spawning of European carp populations starts when water temperature reaches 17-18°C. Females release 100 to 230 g of eggs per 1 kg bw. Eggs are laid on submersed aquatic plants and after contact with water, they become adhesive and swell 3-4 times in volume. Embryonic development takes 60-70 degree-days. Hatched fry stick to substrate and live from yolk supplies. Three days after hatching the posterior part of the swim bladder develops, the larvae start to swim and consume external food of 150-180 µm size (5). Methods of fry production in Asia in hapas, netted channels etc. using natural or artificial substrate (spawning nests), as well as European production of fry in ”Dubravius” or ”Dubisch” ponds are based on this natural process.

Population genetics

The genetic structure of wild populations is very poorly understood. Most phylogeographic and population genetic studies were done on farmed stocks, and looked at differences between the two sub-species, genetic variability within and among populations and the genetic distance among them.

Breeding and culture practices

Production

According to the FAO statistics of 2004 (5), production of farmed common carp was ca. 13% (3,387,918 tonnes) of the total global freshwater aquaculture

production. Common carp production increased by an average global rate of 9.5% per year between 1985 and 2004 and in the past decade (1993-2004), this has increased to 10.4% per year. Asia is the main producing region of the species (China claimed about 70% of the 2005 world production) with the majority of production consumed domestically.

European common carp production in 2004 was 146,840 tonnes, a substantial reduction from the peak production of over 402 000 tonnes in 1990, and reflecting socioeconomic changes in Central and Eastern Europe (Fig.3-4). The European market mostly requires live or freshly dressed fish; processing appears to increase the price to less competitive levels.

Apart from production for human consumption, common carp is produced for leisure as well: i) a significant quantity of the species produced in aquaculture is stocked into fishing grounds for angling purposes, and ii) ornamental fancy varieties, known as Japanese "nishikigoi", are produced for the pet fish market with some prize-winners sold for 10^4 to 10^6 US$, and probably represent the most expensive market of individual freshwater fish (1).

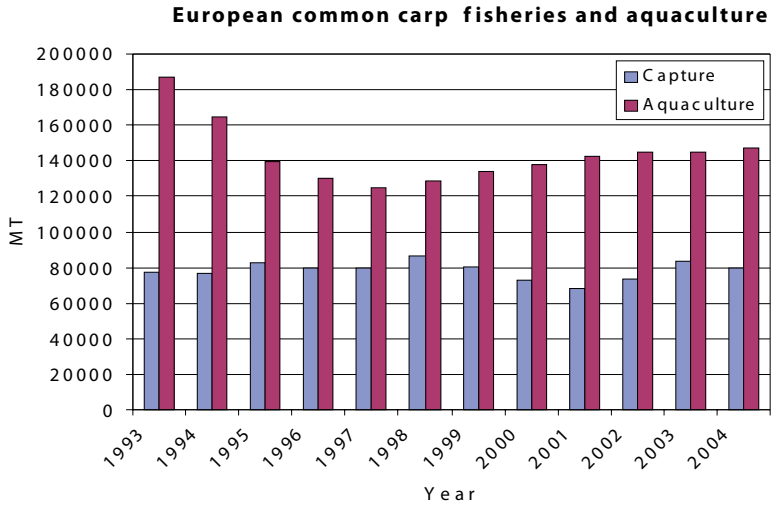

Fig. 3. *Common carp capture fisheries and aquaculture production in Europe (6).*

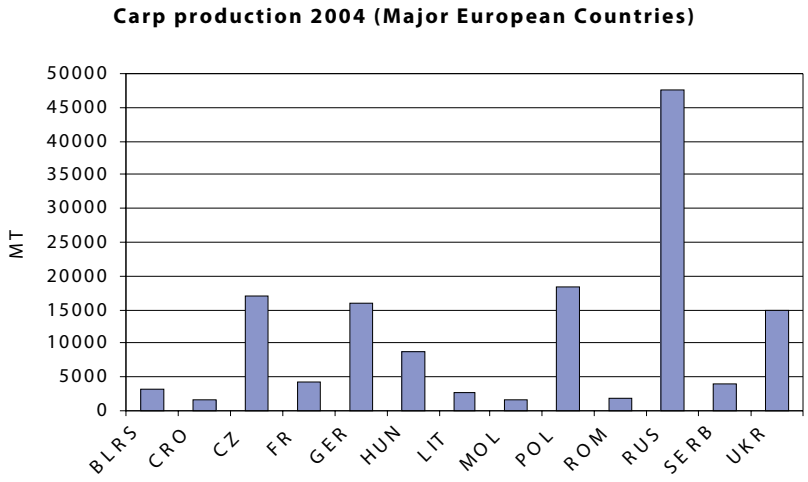

Fig. 4*. Common carp production in major European countries (6).*

Hatchery practices

The majority of production of swimming-up larvae is based on artificial propagation in hatcheries. Broodfish are kept sex-separated in tanks with oxygen-saturated water at 20-22ºC. To induce and synchronize ovulation and spermiation by hormonal stimulants, fish receive injection of pituitary gland, pituitary extract or a mixture of GnRH/dopamine antagonist (7). Gametes are collected by the dry method for immediate fertilization but can be stored for several hours also (8, 9). After gamete activation, the adhesiveness of eggs is eliminated either by the "Woynarovich method" using salt/urea and tannic acid bath, by treatment in milk, or enzymatic treatment (10). Incubation is carried out in hatchery jars. Hatched fry are kept in large trays or conical tanks until stocking at the stage of swimming-up larvae into properly prepared nursery ponds. Approximately 300 000 to 800 000 newly hatched fry can be expected from a single female (5).

Grow-out

The farming cycle in Europe usually consists of the following steps (1, 5). Fry are nursed up to 0.5g bw in shallow drainable ponds in monoculture upon zooplankton with supplementary feeding or in tanks on zooplankton and starter feeds. Fingerling/yearling of up to 30–100g bw are produced in semi-intensive ponds upon manure/fertilizer-generated natural food and supplementary feeding. It can be performed in one step (stocking the swimming-up fry and harvesting fingerlings/yearlings), two-step (stocking nursed fry and harvesting fingerlings/yearlings), or multicycle systems (stocking the swimming-up fry and the fish are thinned out several times). Two-summer-old fish are produced in semi-intensive ponds in monoculture or in polyculture with herbivorous cyprinids, on natural food with supplementary feeding. In temperate climate, one-summer-old fish (20-100g bw) are reared up to 250-500g bw in the second year. Common carp can be reared to market size in extensive or semi-intensive ponds, in monoculture or in polyculture with other cyprinids, tilapias, mullets, etc. on natural food with supplementary feeding; or in intensive systems, in monoculture, on complete feeds (in cages, irrigation reservoirs, running water ponds and tanks, or in recirculation systems). Integrated systems with animal husbandry and/or plant production are also used (e.g. carp-cum-duck in Central and Eastern Europe).

Apart from freshwater predatory fish which may enter the productive systems through water inlets/outlets, the most serious predators are aquatic insects for common carp larvae, snakes and frogs for fry, birds (kingfishers, gulls, terns, herons, cormorants) for fingerlings and yearlings. Mink and especially otter prey on market-size fish and brood fish.

Escapement of common carp can occur from the hatchery (overflow of jars and trays) and from the pond farming systems to open waters (dropped by predators or poachers, draining off the ponds, during floods etc.). Moreover, it is a common practice in some European countries that angling clubs buy stockfish or old broodstock as "trophy fish" from production fish farms and release them into their fishing grounds.

Selective breeding

Qualitative genetic traits studied in common carp included the inheritance of scale pattern ("scaly" SSnn, Ssnn; "mirror" ssnn; "line" SSNn, SsNn and "leather" ssNn) and the lethal/deleterious effects of the N allele (12) , types of pigmentation (wild-type, black, grey, blue, gold, orange, red; 13) used as genetic markers or for selective breeding of coloured breeds, and pleiotropic effects of genes responsible either for

scale patterns or for colouration on various biological and productive characteristics. Compared to fully scaled and/or wild-type coloured carp, those with other scale and/or colour patterns mostly exhibit reduced growth, survival and disease resistance.

Quantitative traits studied involved growth rate, disease- and cold resistance (2, 14) with mostly low to intermediate heritability (h^2) estimates and burdened with environmental biases (2). Selective breeding for disease- and cold resistance resulted in developing several breeds [e.g. Krasnodar carp (15), Ropsha carp (4)], while simple mass selective breeding for growth did not show improvement in the line selected for faster growth (14). Population genetic studies with allozymes and/or microsatellites revealed lower variability of domesticated breeds compared to wild populations (3, 16, 17) and low genetic distance between breeds (3). It indicated that many breeds have been established using small effective number of broodstock, which has resulted in some inbreeding and, which might hamper possibilities of genetic gain from selective breeding (3). With construction of synthetic strains to start a within-strain selective breeding programme with sufficient number of families and standardized family size by separate rearing of families until fry mortality stops, or with parentage assignment by means of microsatellites, more efficient breeding programmes may be designed (3, 18, 19).

Common carp has been subjected to all kinds of chromosomal manipulations (20). Gynogenesis, both meiotic and mitotic, revealed increased homozygosity (with inbreeding coefficients F = 0.6 and 1.0, respectively) and female homogamety XX. Mitotic gynogenesis was used to produce clones. Androgenetic YY males were crossed with normal females to produce all-male progenies. Gynogenetic progeny subjected to hormonal sex reversal resulted in production of XX neomales, and crossing these with normal females produced all-female progenies. Rearing the female monosex stock enhanced production by 7-8% (females being 15% heavier than males), in tropical/subtropical conditions when fish reached sexual maturity before market size. However, in European temperate climate the female monosex stock grew better and had better slaughtering value only in the first three years, but not at market size. Triploids are characterized by reduction in gonad development but not with increased somatic growth.

Microsatellite DNA markers were developed and applied in studies of genetic variation and diversity (21-24), parentage assignment (18) and a genetic linkage map was constructed (26).

Growth hormone (GH) gene transfer was described and the technique was developed to enhance common carp production in China (27), firstly using human GH and later using grass carp GH fused to common carp β-actin promoter. The transgenics showed higher growth performance and food conversion efficiency than the controls, but no transgenic fish have been commercially approved for human consumption (28). Sterile triploid transgenics were produced to avoid environmental impacts.

Most of the world production is carried out using unselected strains (2). When they exist, breeding programmes are mostly based on crossbreeding (14, 29, 30) as it brings quick improvement of growth performance (heterosis effect) in F_1 generation. It is widely used in Hungary, Israel, Czech Republic and other countries. Crossbreeding of breeds developed from both subspecies (*C. c. carpio* and *C. c. haematopterus*) improved survival rate of fry, disease and cold resistance. But improper use of hybrids for further breeding brought contamination to the purebred stocks (31).

Live gene banks of common carp breeds are kept and new forms are continually tested e.g. in the Research Institute for Fisheries, Aquaculture and Irrigation, Szarvas,

Hungary (32), Institute of Ichthyobiology and Pond Culture of the Polish Academy of Sciences, Golysz, Poland, and University of South Bohemia, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic (1, 31).

Interaction studies

Feral populations, some of which could be hundreds of years old, dominate in the majority of the drainage system. Therefore, the status and genetic structure of wild populations are questionable and poorly understood. Phylogeographic and population genetic studies were mainly done on farmed stocks, and only occasionally involved «wild» stocks. Nothing can be said about local adaptation, given the status of wild populations.

The basic biology and distribution of the common carp, and especially of farmed stocks, is well understood.

Being a benthic feeder, carp typically make the water turbid. There is evidence from countries to which carp was introduced (e.g. Australia, USA, Mexico) that increased turbidity caused by carp has negatively affected local fish communities as well as vegetation which prefer clear water. Apart from competition for food and habitat, we are not aware of other direct negative ecological effects of carp. Since carp has been introduced to almost all European countries, and established in open waters, potential damage has already been done. Since carp is regarded as a premium angling species in many countries, measures should be taken to prevent further release into new open waters, and stocking for recreational purposes should be limited to closed water bodies.

Conclusions / Implications

- *Preservation of biodiversity*. A comprehensive biodiversity survey, aiming at identifying wild populations (native as well as introduced) is urgently needed. It should be carried out in cooperation with local fisheries agencies and/or experts in each country, and should include documentation (including genetic analysis using modern genetic tools to delineate genetic relationships among wild stocks), as well as cryopreservation of semen samples. It is also recommended that areas where wild stocks still exist will be declared as sanctuaries to preserve those apparently rare wild resources. These wild populations should be subjected to detailed investigations of life history traits, including reproductive strategies, fecundity, survival and fitness under variable environmental conditions, e.g. pH, temperature regimes, etc., in attempt of identifying local adaptations.

- *Breeding programmes*. A survey of farmers' needs is required to identify breeding goals and to make sure an improved stock will be well accepted (in view of the traditional preference of locally developed purebreds in some countries). A national or European family-based selective breeding programme should be started to meet those needs.

Dissemination of the improved breed should be done carefully, making sure it does not affect the rare wild resources that may still exist. Biocontainment methods should be applied.

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European sea bass - *Dicentrarchus labrax*

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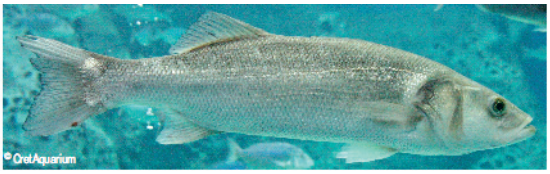
Biology, ecology and genetics



Distribution

The European (or common) sea bass, *Dicentrarchus labrax* L. (Moronidae, Perciformes) is found in coastal waters of the Atlantic Ocean from South of Norway (60°N) to Western Sahara (30°N) and throughout the Mediterranean Sea and the Black Sea. It has been introduced for culture purposes in Israel, and more recently in Oman and the United Arab Emirates.

Capture

It is a fish with high commercial value both from capture from wild stocks, and in the last 25 years from aquaculture production. In 2004, the global sea bass capture fisheries production was of 11,481 tonnes (1) with France and Italy accounting respectively for 42% and 29% (Fig.1).

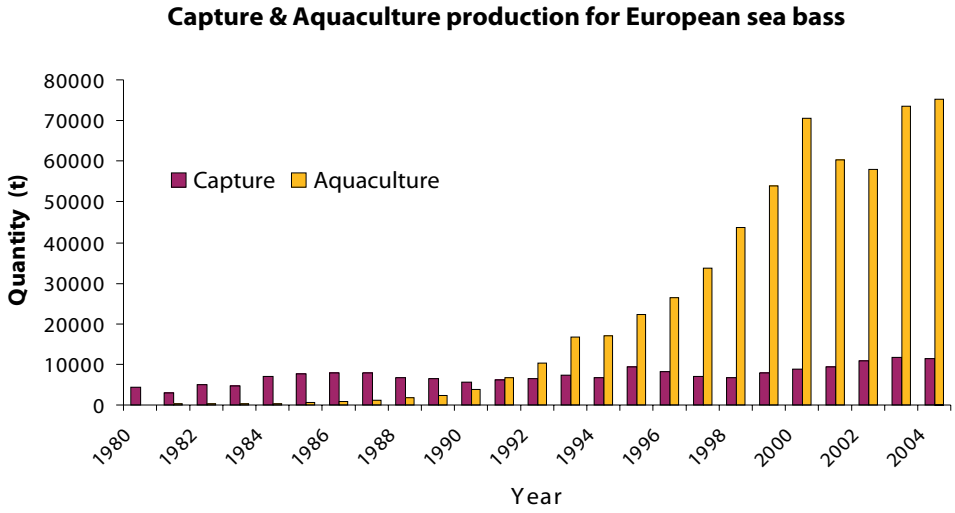


Fig. 1. *Capture fisheries (1) and aquaculture production (2) for European sea bass in Europe and in the Mediterranean*

Biology

European sea bass is a gonochoristic species. Females spawn in winter in the Mediterranean Sea (December to March) and up to June in the Atlantic Ocean. They present a high fecundity (on average 200 000 eggs / kg of female), start to reproduce over 2 kg and can reach 6 to 7 years in the wild (3). Eggs and larvae have a great dispersal during the 3 first months of life and adults migrate over several hundreds of kilometers.

Population genetics

European sea bass population differentiation is one of the best studied among European marine fish. There are numerous genetic studies based on allozymes, mitochondrial

DNA or RAPDs (4-8), microsatellites (9-14) at different geographic scales. These studies have led to the identification of three genetically distinct zones: the north-eastern Atlantic Ocean, the western Mediterranean and the eastern Mediterranean. On the basis of microsatellite loci the transition zones have been localized at the Almeria-Oran oceanic front between the Atlantic (the Alboran Sea included) and the western Mediterranean and somewhere around the Siculo-Tunisian strait between eastern and western Mediterranean. This was surprising for this euryhaline and eurythermic demersal species, since adult migratory behaviour has been reported as reaching several hundred kilometres. Nevertheless, this might be related to climatic changes and sea water fluctuations during the Pleistocene that undoubtedly had a strong influence on the distribution of the species in the Atlantic Ocean and the Mediterranean Sea.

There was no significant population structure found either in the Atlantic or in the western Mediterranean. On the contrary, the genetic structure of eastern Mediterranean sea bass populations is consistent with the subdivision of the region into several basins, e.g. the Adriatic, Ionian and Aegean Seas, the Libyco-Tunisian Gulf and the Levantine basin (13). Cases in which populations do not cluster with other samples belonging to the same geographical origin are not surprising, since eggs or fingerlings originating from the western basin were most likely used to seed many hatcheries around the Mediterranean, when sea bass aquaculture began.

It was suggested that some allozyme loci in *D. labrax* exhibit patterns of allele frequencies shaped by adaptation in different environments. When data from microsatellite and allozyme markers in Mediterranean lagoon and marine populations were compared (14), there was evidence that half of the allozymes used in the analysis undergo some sort of selection and only few allozyme loci seemed to be implicated in the differentiation between marine and lagoon samples.

Breeding and culture practices

Production

Aquaculture production reached 80 161 tonnes in 2005 (2), with Greece producing 35 000 tonnes, followed by Turkey (20 900 t), Italy (9,800 t), Spain (6 130 t), and France (4 300 t).

Hatchery practices

Eggs are produced all year around using adequate temperature and photoperiod. Sea bass spawn naturally in tanks and buoyant eggs are collected at the water outlet of the spawning tanks. In captivity, first sexual maturation occurs in 1-2 years-old males and in 3-5 years-old females. A generation interval of 2 years can be obtained in controlled rearing conditions but in practice it is longer and ranges between 4 and 6 years. Eggs and sperm can be collected by a gentle pressure on the flanks of anaesthetized fish. Hormonal stimulation of ovulation by LHRH-a or GnRH-a is needed to collect eggs (15). Sperm can be frozen with several types of protocols.

Grow-out

Most of the sea bass production is achieved in sea cages at 10 to 20 kg/m^3. Sea bass is also reared in concrete raceways in France, Italy and Spain (30 to 80 kg/m^3), in ponds (in extensive systems in Portugal, Greece and Egypt, < 2 kg/m^3) or in closed systems. Restocking of juveniles in lagoons is traditionally performed in a limited way in Greece and in Italy. The genetic impact of these releases is uncertain. Farming has been in progress since the mid 1980s (20) when the mastering of early survival until weaning (from 0 - 10% to 35 - 65%) was achieved with better management practices. The fry quality variability remains one of the main issues. Other important issues are related to

the species slow growth, its susceptibility to viral diseases in warm waters and to its poor conversion efficiency (>1.6 in large size fish). Some of these critical aspects can be linked to the fact that in farming conditions there is an excess of males (70 to 95%) that present a precocious sexual maturation (<100 g) and a slower growth.

Selective breeding

Domestication of the European sea bass has been initiated in the mid 1980s by some pioneering companies in France, Spain, Italy and Israel and some strains are now kept in captivity and selected since 5 to 6 generations. However, most of the hatcheries still maintain their own broodstocks, often scarcely recruiting from wild populations or with juveniles bought in the market. Deviations from HW equilibrium are documented in some hatchery populations compared to wild ones, mainly as heterozygote deficiencies (6, 10, 13), less alleles, and differences in allele and genotype frequencies. This may be due to mass selection practices, the addition of F1 or F2 individuals to the original breeders' pool or small founder effects. There were also cases in which some of these aquaculture stocks were found largely outbred and open to fishes coming from the wild (12).

Only two studies report the performance evaluation of different farmed strains (16) although differences in growth performance in seed originating from different hatcheries are well known and recognized by growers. A first evaluation of genetic parameters for growth has been reported (17) indicating the real potential of selection to efficiently improve this trait. Recent advance achieved in the EU CRAFT project (18) has also provided first genetic parameters for quality traits, sex determinism and genotype*environment (G*E) interactions. All the measured traits (gutted yield, fat in the fillet, filet yield, sex–ratio) present an intermediate to high additive genetic determinism indicating that genetic progress by selection can be obtained quickly. A low level of G*E interaction was quantified for all the traits in the different environments tested (pond, cage, race-way, re-circulating system).

Several selective breeding programmes have been initiated in France, Israel, Greece and Spain (19, 20). Traits selected are growth, morphology or carcass yield. These programs will likely play a major role for dissemination of genetically improved seed in the future. The high fecundity of the species allows high selection intensity and facilitates rapid genetic gain. It is assumed that as much as 80% of the sea bass production comes currently from commercial populations which have undergone some level of genetic improvement. In some cases, DNA fingerprinting is used in order to optimize mating schemes and limit inbreeding. At least two commercial laboratories propose their services to perform parentage assignment for this species using fingerprints.

Females are 10 to 40 % larger than males. The farming of all female populations is one of the potential option to increase the overall production efficiency. According to the EU regulation (Directive 96/22/CE), the phenotypic sex of the breeders can be controlled by hormone administrated in the diet after weaning and 100% of males or females can be achieved depending on steroids used . Sex ratio was proven to have a genetic variability between sires, a positive genetic correlation with growth and an interaction with temperature was also found (21, 22). However, applied and economically beneficial protocols are lacking for the industry.

The species has 48 chromosomes. Triploids have been produced using different protocols and show a gonad sterility in females and gamete sterility in males. Growth is equivalent or 20 % lower than diploids growth. Nevertheless, triploid females' growth is almost the same than that of diploid males indicating that farming of such genotypes could be seen as a potential application to limit genetic risk potentially associated with escapees (23). Several other traits remain to be evaluated such as feed conversion efficiency during the reproductive season, disease resistance and performance in cages. Triploids are not

grown by the farmers, for the fear of consumers and citizens reaction. It seems obvious that this technology will need to be associated in the future to the increase of female proportions by the use of environmental and/or genetic monosexing. Gynogenetic and homozygous clones were produced. Trials to produce tetraploids were not successful and no successful transgenesis is reported in sea bass in the literature.

Genomics

European sea bass is gradually heading towards being one of the ten most genome rich teleosts. An EU funded project (24) produced a first generation linkage map with more than 250 microsatellite markers, which were added to those previously reported (25), few hundred ESTs, more than 200 AFLP markers (to be included in a 2nd generation linkage map) and a 6X coverage BAC library. QTL mapping of 14 commercially important features of sea bass is in progress. Since 2004, three other European projects (26-28) include sea bass genomics; in the context of the NoE Marine Genomics Europe (MGE), several cDNA libraries were constructed and a medium-scale EST sequencing project has been completed with more than 17 000 EST's, in which hundreds of SNPs and sequences containing tandem repeats (SSR-ESTs) have already been identified. Finally, Radiation Hybrid (RH) panel and BAC-ends sequencing projects are under way moving towards the construction of a physical map.

Interaction studies

The entity of escapees is unknown. Escape events can theoretically occur at all the stages of the farming process: induced breeding, larval stage, grow-out. It can also occur during transport of fish from the hatcheries to the growing sites and from the growing sites to the processing plants. Risk of escape from the hatcheries seems limited as most of them operate in closed systems with effluent treatment.

Most of the data are mainly based on limited studies and may not precisely describe the current situation and thus the potential risks associated with mid- and long-term interaction between wild and farmed populations today. A phylogeographic analysis based on microsatellites (13) revealed that among the wild eastern samples, two samples from Greece and one from Egypt did not cluster according to their geographic origin, but rather with the western Mediterranean group. Furthermore, a wild population from the Gulf of Tunis, although clustered within the western Mediterranean group, probably originated from aquaculture. Within these particular samples, a lower allelic diversity was observed, indicating that they originated from a limited number of broodstock of foreign origin. These findings may be explained by the use of Western fingerlings to seed Eastern Mediterranean farms in the early 1980s, and escapes in the wild.

Conclusions/Implications

Future work should include the genetic analysis of additional natural populations, mainly from the eastern Mediterranean where sea bass aquaculture production is the largest. In principle, there is no sound reason to suspect a reproductive barrier between local and domesticated stocks and the persistence in the wild of stocks genetically identified as of 'western origin' could imply the existence of behavioral (e.g. assortative mating) and/or physiological (e.g. shift in the reproduction period) mechanisms that limit interbreeding and should also be the subject of further study. Future works should also include evaluation of domestication effect on the fitness of domesticated fish and its ability to survive and breed in the wild.

It is obvious from genetic studies that the European sea bass consists of well-defined stocks throughout its distribution range. Practices such as the crossing of different strains

and the eastward transfer of broodstocks, eggs, larvae and fry over large distances can involve the risk of causing artificial gene flow from escapees to local populations; this could induce a biodiversity decline or outbreeding depression.

The distribution, abundance, temporal change and life cycle (early survival, migration, and reproductive behavior) should be investigated to get a more precise view of how escapees can interact with their wild congeners. Moreover, the genetic impact of escapees is not known at all. Several factors can explain this lack of knowledge:

- The biology and the ecology of the European sea bass in the wild are not well documented regarding key aspects such as the juvenile phase or the species migration. Moreover, populations and sub-populations in the eastern Mediterranean and at the limit of the Atlantic distribution of the species (Morocco to Norway) need to be analyzed.
- The application of selective breeding in captive broodstocks is recent, and it can be assumed that the genetic differentiation between wild and farmed populations can be limited.
- Selective breeding may have already induced some change of fitness traits in the wild, which is expected. This may already have reduced the ability of domesticated fish to succeed to interbreed with wild congeners; however, this needs to be addressed.
- Selective breeding programmes depend not only on technical factors but also on business decisions, fusion, acquisition, concentration, stock exchange value. This changing and unpredictable evolution should be taken into account in the future in order to set up efficient tools to evaluate the genetic impact of escapes.

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Gilthead seabream - *Sparus aurata*

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Biology, ecology and genetics

Distribution and capture

The gilthead seabream, *Sparus aurata*, is a subtropical Sparidae distributed from 62°N - 15°N, 17°W - 43°E. It occurs naturally in the Mediterranean and the Black Sea (rare), and in the Eastern Atlantic, from the British Isles, Strait of Gibraltar to Cape Verde and around the Canary Islands (1). Gilthead sea bream are captured with traditional and sporting equipment, and sometimes with semi-professional systems (Spain, Sicily, Egypt and Cyprus); trawl nets, bottom set longline and hand line are commonly used. This species is regularly present on the markets in the Adriatic Sea, Greece, Turkey and the Maghreb. It is commercialised fresh, refrigerated and frozen. In 2004, the world capture production of gilthead sea bream was of 8.914 tonnes, i.e. less then 10% of aquaculture production (2).

Biology

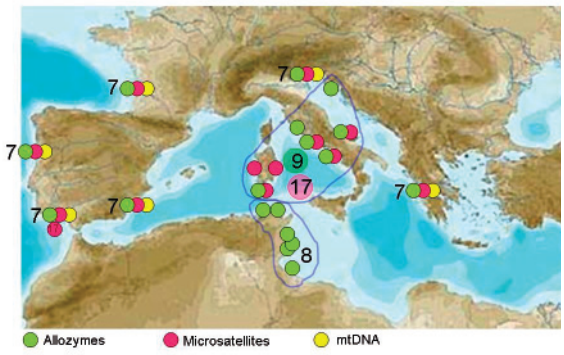
The gilthead seabream inhabits seagrass beds and sandy bottoms as well as the surf zone, commonly to depths of about 30 m, but adults may occur at 150 m depth. It is reported as a sedentary fish, though migrations are likely to occur on the Eastern Atlantic coast, from Spain to British Isles. It occurs either solitary or in small aggregations. It is an euryaline species and moves in early spring towards protected coastal waters in search for abundant food and milder temperatures (trophic migration). In late autumn it returns to the open sea for breeding purposes, being very sensitive to low temperatures (lower lethal limit is 2°C). It is mainly carnivorous (shellfish, including mussels and oysters), accessorily herbivorous (1).

The sea bream is a protandrous hermaphrodite: it is a functional male in the first two years and at over 30 cm in length becomes female. During the male phase, the bisexual gonad has functional testicular, with asynchronous spermatogenesis, and non functional ovarian areas (3, 4). Ovarian development is also asynchronous, and females are batch spawners that can lay 20 000-80 000 eggs per day for a period of up to 3 months. In the Mediterranean, they reproduce between October and December. The eggs are spherical and pelagic, with a diameter slightly lower than 1 mm and a single large oil droplet. The planktonic larval stage lasts about 50 days at 17-18° C.

Population genetics

Studies on gilthead sea bream have been carried out through gene-enzyme systems (5-10), AFLP (11) and mtDNA (7, 12, 13) analyses. In recent years, approximately 25 microsatellite loci have been reported (14-16) and several studies (6, 7, 11, 17, 18) have been performed by using these molecular markers. Additional 200 microsatellites and more than 3 000 ESTs (Expressed Sequence Tags) have recently been produced within Bridgemap, an EU funded project (19).

Until a few years ago very little was known on the genetic structure of *S. aurata* and the first studies reported conflicting data concerning the existence of panmictic (5) or subdivided populations (12). More recent studies (7, 8, 9, 17, see figure for sampling localities) have depicted a picture of species subdivision that still needs to be clarified. A strong differentiation has been detected through allozymes between samples from the Tunisian coasts (8), but only a slight, though significant, genetic differentiation has been detected on a large-scale area, in several wild sample sets from the European Atlantic and Mediterranean coasts, both through allozymes (7, 9) and microsatellites (7, 17). The allozyme (21 loci) analysis of *S. aurata* from six localities along the Tunisian coast evidenced population sub-structuring (total F_{ST} = 0.093, highest pairwise F_{ST} = 0.265) in southern and northern populations, with a strong geographic trend (8). The analysis of gilthead seabream from three Atlantic and nine Mediterranean localities with a total of five different microsatellite loci and 37 different enzymatic loci, produced considerably lower F_{ST} values, similar in different studies, i.e., F_{ST} = 0.031 (7) or F_{ST} = 0.017 (9) for allozymes and F_{ST} = 0.036 (7) or F_{ST} = 0.010 (17) for microsatellites, suggesting a lower, but still significant, genetic differentiation and a weaker structuring pattern. Though some main assemblages (9, 17) can be identified, reflecting the collecting areas, the pattern of population subdivision is not reconcilable to an isolation-by-distance model.



Genomics

Since 2004, two European projects, Marine Genomics Europe (MGE) (20) and Aquafirst (21), have the ambition to render the genome of the gilthead sea bream as one of the best characterised among teleost species, apart from classical model fishes. Through the MGE data production pipeline (20), a medium-scale EST sequencing project has already been completed, with more than 18 000 ESTs from several sea bream tissues, in which hundreds of single nucleotide polymorphisms (SNPs) and sequences containing tandem repeats (SSR-ESTs) were identified.

The 200 microsatellite markers and the 3 000 ESTs produced within the Bridgemap project (19) lead to the construction of a first-generation genetic linkage map (22) and an RH map (23). Comparison between the two maps reveals a good concordance, as all markers in a single linkage group (LG) are also located in the same RH group. Moreover, a parentage analysis and a pilot QTL analysis are in progress toward the identification of genetic loci involved in the determination of economically important traits.

Breeding and culture practices

Production

The gilthead seabream has traditionally been cultured in Mediterranean coastal lagoons and brackish/salt water ponds, especially in the northern Adriatic *valli* in Italy and the Egyptian *hosha*. These extensive fish rearing systems acted as like natural fish traps, taking advantage of the natural trophic migration of juveniles from the sea. Restocking was usually performed with wild fry and juveniles, collected by specialised fishermen. By the late 1970s, the reduced availability of wild fry and the increasing demand from intensive farms enhanced the development of induced spawning techniques, establishing by the end of the 1980s a production scheme based on a reliable and programmed quantity of fry (24).

Sea breams are farmed extensively in lagoons, or intensively in tanks or cages. At present, most production is from intensive farming, with average densities of 20-100 kg m^3 and a FCR is 1,5-2: 1. Extensive farming still remains a traditional activity in some regions, but with a very low impact on the market. In 2004, the global aquaculture production was of 90,995 tonnes (Fig. 1). In the Mediterranean the main producers are Greece, Turkey, Spain and Italy (Fig. 2). At the beginning of the 1990s, twenty sea bream hatcheries were operating in the Mediterranean; at present over 65 hatcheries are distributed in Croatia, Cyprus, France, Greece, Italy, Morocco, Portugal, Spain and Tunisia.

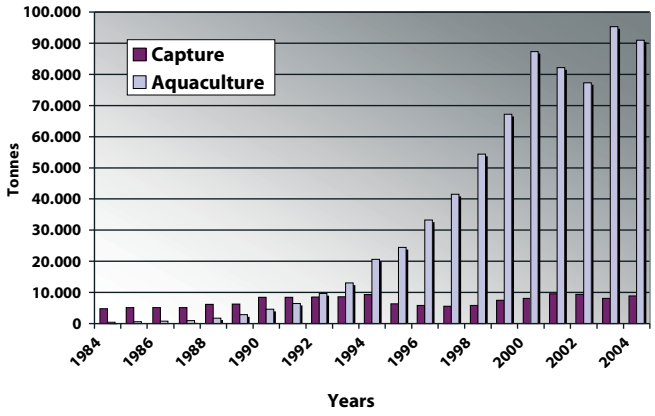


Fig. 1. *Global capture fisheries and aquaculture production of sea bream (2)*

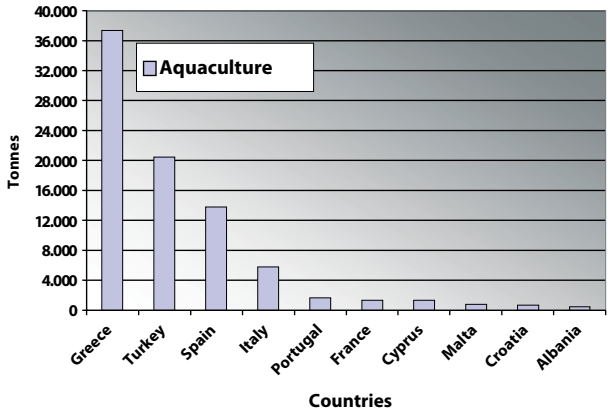


Fig. 2. *Major aquaculture producing countries in the Mediterranean in 2004 (2)*

Hatchery practices

At first, broodstocks were all from the wild, with a substantial division among two different stocks, the Mediterranean and the Atlantic ones. Today, after twenty years of hatchery practices and, most importantly, fry market all over Europe and the Mediterranean, strains are mixed and even the large distinctions among Mediterranean and Atlantic stocks are dispersed. Most broodstock are kept for spawning for several years. New males must be added to the broodstock every year, as they turn into females at 2 years old, so that a 5 to 20 % per year renewal occurs, whenever possible with wild fishes. However, often males are taken from F1 and farmers select among their stock the best performing specimens for reproduction. One female can produce up to 1 million eggs and the normal fertilization ratio is 90 – 95 %. Fertility and egg quality are strictly related to a calm environment and a balanced diet. Males range around 150 – 300 g, while the optimal female weight is around 1,5 kg. Hormones (HCG and, later, luteinic hormones) were used at

first to induce spawning, then replaced by modulation of environmental rearing conditions, in particular temperature and photoperiod, that enabled to extend the spawning season to all year round. Larval rearing is performed in cylindroconical tanks 3 to 6 m in diameter, at a stocking density of 100 to 250 individuals per liter and first feeding starts at day 4 (at 19 °C). Green water is the most used system and larvae are fed with enriched rotifers for 25 days, then artificial feed and Artemia nauplii, and finally artificial feed only.

Optimization and control of the major environmental parameters have been the first steps to improve fry production, in particular temperature, salinity, dissolved oxygen, light intensity and photoperiod (25, 26). The fry quality is directly linked to the later performances in grow out. Morpho-anatomic quality principally refers to frequent malformations affecting swim bladder, opercula and skeleton. The genetic origin of such anomalies has not been demonstrated, and is presently being studied within an EU project, FINE FISH (27).

Large volumes technique is an alternative technique for fry rearing, mainly performed at experimental level, and conjugates the green water and semintensive mesocosms techniques. Its use is under debate, especially as far as the number of malformations is concerned.

Restocking programmes

In Italy, Greece and Spain restocking of coastal lagoons with wild fry of gilthead sea bream has been carried out for many years, in order to increase the production in these confined brackish water bodies. Specialized fishermen (in Italy called *pescenovellanti*) had fishing rights for a definite site on the coast line. After capture, fry were then carefully transported from all over Italy to the *valli* and coastal lagoons for restocking purposes, paying no attention of fry origin. Today restocking is performed mostly with hatchery fry, though broodstock origin is seldom known. Since 1994, more than 1 100 000 juveniles have been released in various Greek lagoons (24). In Orbetello Lagoon (Central Italy) restocking with hatchery fry started in 1995 and the production of gilthead sea bream increased 9 folds from 1995 to 2000. Sea ranching is not performed in the Mediterranean.

Selective breeding

A leading livestock company in Greece initiated a large scale family based selective breeding programme for sea bream in 2002 (28). The base material was collected broadly from locations in Greece and elsewhere, and 50 full- and half-sib families were produced and performance tested annually. Re-use of a limited number of breeders in subsequent year-classes allows for testing of approximately 150 families per generation. The estimated selection response for growth rate resulting from the first cycle of selection was approx. +20% (Rye, pers. comm.). The first genetically improved eggs were marketed in 2005. Selective breeding programmes are also carried out in France.

The principal objective of a new EU project (29) are to identify genes whose expression is associated with disease and stress resistance and, from this information, to develop genetic approaches that should allow characterisation of genetic markers for marker-assisted selective breeding of disease and/or stress resistant individuals. Therefore, a new QTL analysis in the species will be carried out and the density of the sea bream genetic map will be increased with the addition of more markers to determine the relative map position of candidate genes.

Interaction studies

Little is known on the success of restocking practices and their impact on wild local populations. Similarly, very few data are available on the escapes of fish from accidental cage breaks or poor management practices, as there is no legislation/ regulation requiring mandatory reporting of these events. The intentional release of fry of unknown origin in restocking programmes in more or less confined coastal lagoons, or the accidental escape of fish from farms, have certainly contributed to a mix of all gilthead sea bream genetic stocks. This is particularly important in the last decades, with the development of transport means which enabled long distance transport of eggs, fry and broodstock.

Genetic analysis (30) of 13 Italian broodstocks revealed that the number of micro-satellite alleles was not significantly different from those from natural populations and no decline of genetic variability parameters has been observed. However, geographic assignment of breeders (18) revealed a mixed and highly heterogeneous origin of broodstocks, with a high percentage of Atlantic individuals among breeders, and a significant genetic divergence between cultured samples and local wild populations (7). On the other hand, parental assignment of offspring from several mass-spawning events (31) demonstrated the consistently low effective population size, and the influence of the differential male and female average contribution, thus evidencing the needs to increase effective population size in order to manage the genetic variation within the farm population. A simulation study (11) demonstrated that molecular tags (AFLP, microsatellites) allow the identification of hatchery escapees of both Atlantic and Mediterranean origin among wild fishes. Hence, genetic tagging of sea bream broodstocks in commercial hatcheries might be a suitable tool to monitor the genetic impact of fish farm escapes/releases.

Conclusions/Implications

There is a first need to investigate on the life cycle and ecology of the species in the wild, as little is known on its biology, in particular the effective and census sizes, spatial distribution, spawning grounds and behaviour, and eventual migration. This basic biological information is needed to understand how the farmed individuals, depending on the life stages and places where they are intentionally or accidentally released, may interact with the wild ones.

The species subdivision within the Mediterranean Sea, suggested by all studies, discloses a potential impact of aquaculture on natural populations. Indeed, the common practice for farmers to use breeders of different geographic origin could determine changes in allelic frequencies and/or the introduction of non native alleles into local populations in case of escapees from culture facilities, in particular from floating cages, or restocking programmes.

As molecular genetic markers represent a suitable tool for genetic tagging of both wild, broodstock and cultured gilthead seabream (11, 18, 20-23, 29-31), it is urgent to genetically investigate additional natural populations, in order to cover the whole geographical range of the species and to fill the sampling gaps (even using historical collection of samples - scales, otoliths, pickles), to i) qualify the species fragmentation in terms of the genetic differentiation underlying it; ii) extend the baseline for more sensitive monitoring tool and iii) investigate whether the molecular markers are connected to peculiar biological traits in different regions of the species distribution range.

As far as hatchery broodstocks are concerned, further investigation is required in order to determine broodstock origin and entity in all main hatcheries, their subdivision in different spawning stocks and their genetic variability. Consequently, it would be important to trace, record and quantify "gene flow" in the industry through the exchange of fry and broodstock among producing countries.

Last, studies should be performed to determine to what extent spontaneous spawning, and consequent egg and fry release, occurs in cages (an event which has been reported by farmers) and to develop technical solutions to eliminate it.

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Turbot - *Scophthalmus maximus*

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Biology, ecology and genetics

Distribution

The turbot, *Scophthalmus maximus*, also called *Psetta maxima* (Scophthalmidae, Pleuronectiformes), is naturally distributed in European waters, from Northeast Atlantic to the Arctic Circle (30º to 70ºN; 23ºW- 42ºE). It occurs in the Baltic and in the Mediterranean, as well as in the Black Sea, where a subspecies *Psetta maxima maeotica* has been described (1). It also exists in the Southeast Pacific Ocean (Chile) and in China, where it has been introduced for farming. Wild populations inhabit along all European coasts to North West Africa (Morocco), where it is also farmed. Clear geographical discontinuities have not been reported between populations. Though this species is not considered endangered, declines in wild catches (2) and some genetic evidence (3) suggest the existence of historical population reductions for European turbot.

Capture

With some marked oscillations during the 1980s, turbot catches yield more than 7 000 metric tonnes annually (Fig.1). In the last years a considerable proportion of total production derives from aquaculture (more than 35%), principally in Spain and France.

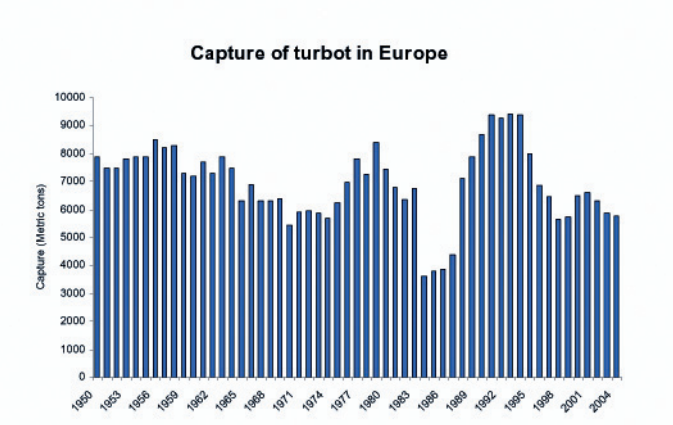

Fig. 1. *Turbot capture fisheries in Europe (4)*

Biology

The turbot is a predator species that lives on sandy, rocky or mixed bottoms; it is common in brackish waters. When juvenile, its diet is based on crustaceans: Malacostraca and Decapoda. Adults feed mainly on other bottom-living fishes (sand-eels, gobies, etc.), and, to a lesser extent, on crustaceans and bivalves. Its trophic level,

estimated from a number of food items using a randomized resampling routine, is 2.8. The turbot exhibits one of the most important growth rates observed in flatfish (around 30 cm every 3 years).

It exhibits a medium resilience, with a minimum population doubling time of 1.4 - 4.4 years (K=0.15-0.28; Fecundity=5 million). Juveniles migrate short distances, less than 10 km in the first two years of life as shown by tagging-recapture in Danish waters (5). Their migration seems to be related to tidal cycles in nursery grounds and is also associated to foraging activity. This migration pattern is followed by an increase in the offshore migration distance, likely associated to spawning behaviour.

The spawning season occurs between April and August in Mediterranean populations and between May and August in Atlantic areas. Females reach maturity at 3 years old (around 46 cm length) and males at 2 years old (around 30 cm long). Fecundity is generally over 5 million eggs. Their eggs are pelagic, smooth and spherical, of 1.1 mm diameter and an oil globule of 0.18 mm.

Population genetics

With 2n=44 chromosomes, its karyotype has been described and many chromosomes characterized by differential banding (6). There are various polymorphic genetic markers available for turbots. Variability at 17 allozymes has been described for wild European populations (7, 8). Some microsatellite loci are available in publications (3, 9, 10, 11, 12) and in the GenBank databases. Population variation at mitochondrial DNA sequences (control region) has also been described (13).

Very little is known about population structure in wild turbot. In the wild, most analyzed populations are in Hardy-Weinberg equilibrium at all loci (coding and non-coding), indicating panmixia (at random mating). In Europe, no genetic differentiation was detected between populations. It was not detected with allozymes (7), neither employing more polymorphic markers such as microsatellites; for example between Atlantic and Cantabric populations (3), and between much more distant populations such as Ireland and Norway (9). Most of the genetic variance is distributed within samples. Genetic differentiation between neighbouring populations has been reported only in one case, likely associated to physical barriers (14). In general, high levels of gene flow seem to exist for this species, as for other marine species. Low population genetic diversity at allozyme loci, compared to other flatfishes with similar habitat and life history features like brill (*Scophthalmus rhombus*) and flounder (*Platichthys flesus*), suggests historical population bottlenecks along turbot evolution (3). Discrepancy with high microsatellite variation has been explained by differential mutation rates. This hypothesis, however, has not been fully explored in turbot. More markers (mitochondrial and nuclear) should be used to analyse more populations in a wider geographical extent.

Intense gene flow between regions does not exclude the existence of genetically distinct lineages in some geographical areas. The Mediterranean turbot, for example, consists of two main genetically different lineages, one western and one eastern (13). Significant differentiation between North Sea and Baltic Sea turbot has also been described based on microsatellite loci variation (14). Multiple hybrid zones in the transition area between both seas have also been evidenced. Thus it is possible that other differences associated to geographic location appear when genetic studies are expanded for this species.

A sharp cline in genetic differentiation going from the low saline Baltic Sea to the high saline North Sea (14) suggests local adaptation to salinity conditions in these regions. Salinity conditions for optimal spawning vary between North Sea and Baltic Sea

turbot. However, scarce information exists about this point for wild turbot in other areas. There are no other published studies of fitness in wild turbot populations. It is the only known case of sharp genetic differentiation likely associated to adaptation to different environmental conditions. However, at present nothing is known of the genetic architecture of traits associated with adaptation of turbot to different environments, although they are likely polygenic as other ecologically important traits in other fish species.

Breeding and culture practices

Production

Aquaculture of turbot first started in Scotland in the 1970s. At the beginning of the 1990s the technological development of juvenile production allowed to expand considerably the production of aquaculture turbot and the number of farms. Turbot is cultured in Spain, France and Portugal but also in Denmark, Germany, Iceland, Ireland, Italy, Norway and Wales. European production has now stabilised around 5 000 tonnes per year. Spain is undoubtedly the world leader of adult turbot production (50% of the total production). Nowadays, fishery and aquaculture share almost equally the market.

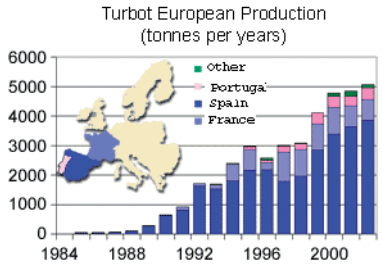

Fig. 2. *Turbot aquaculture production in Europe (15)*

Hatchery practices

Broodstocks are based on both wild and farmed individuals. Breeders are maintained in concrete or cement squared tanks (density: 3-6 kg/m^3) and fed on moist pellets. Turbot do not spawn spontaneously in captivity, thus gametes must be hand-stripped (16). As in the wild, females exhibit significantly higher growth rates than males and reach sexual maturity earlier. Males produce poor sperm in terms of both quality and quantity compared to other marine Teleosts, but females can produce 5 to 10 million eggs. Embryonic development takes 60-70 days. Spawning can be obtained all year round, modifying rearing temperatures and day-night rhythms (17). Hormones treatments can also be used to manage advanced spawning in broodstock and to obtain egg production all year round (18). Larvae survival is generally lower than in other cultured marine fishes. Newly hatched larvae, 60-70 days after fertilization, are 2.7-3.1 mm long. Larval rearing can be intensive (15-20 larvae/l) or semi-intensive (2-5 larvae/l) in tanks with open-circuit pumped seawater. At the beginning of the exogenous feeding stage (about 3 to 7 days after hatching), larvae are fed rotifers (*Brachiomus plicatilis*), *Artemia* nauplii and phytoplankton. Metamorphosis ends after 40-50 days when larvae are about 25 mm long. During 2-3 months after hatching, fishes are nursed in small tanks and start to be fed with dry pellets until they reach 5 g weight.

Grow-out

Most commonly, turbots are reared in circular concrate tanks with open-circuit pumped seawater (or increasingly in re-circulation systems). They can also be grown out in flat-bottomed metal cages (cages floating or submerged at various levels in the sea). Despite reduced production costs, culture at sea remains less used as it is not easy to find sites with adequate environmental conditions for optimal growth (e.g. temperatures around 16°C).

In both rearing techniques (land-based and at sea), turbot are fed with extruded pellets and sometimes with fresh food (fishes). Turbot tolerate overcrowding, thus stocking density can reach 100 kg/m². Commercial size is around 1.5-2 kg. Productivity of turbot culture depends mostly on fry quality, rearing temperatures and on the control of the main pathologies affecting captive individuals (vibriosis and furunculosis). Environmental factors favouring optimal growth in turbot are now well known (19, 20).

Selective breeding

Adaptation to farm environment has been suggested in this species. Cultured turbot are heavier than wild fish of the same age and length (21) and show very high incidence (96%) of abnormal lateral line canals and development differences during the larval stage. There are also some evidences that turbot farmers are selecting individuals with high growth rates to increase production and skin pigmentation.
Early sexual maturation before marketable size usually occurs in males, but also sometimes in females, compromising further growth. Consequently, turbot producers are interested in techniques allowing (i) to produce sterile stocks by polyploidisation or (ii) to obtain all-female stocks. Induced triploidy in turbot has been recently published (21). Experimental polyploidy (i.e. increased number of chromosome sets induced by altering meiotic or mitotic divisions) can produce total or partial sterility. This boost growth, prevent changes in skin color, and maintain meat quality. In turbot, induction of triploidy can be obtained by applying cold shocks shortly after fertilization (22). The growth increase observed in triploids turbot remains however relatively low (19).

Turbot show female homogamety. As females reach higher weight (10 to 20% after 800 g) and mature later, it would be advantagous, in the future, to use female monosex progenies in turbot aquaculture. Gynogenesis induction (production of offspring with exclusive maternal inheritance), one of the first steps towards the production of monosex female stocks, is already possible in turbot (23).

Information about genetic monitoring of broodstocks and cross schemes is very scarce for turbot. In the only study published to date, there is evidence of strong family structure (high coefficients of relationship between individuals) in domestic strains (3). As fecundity is very high in this species, crosses in aquaculture stocks probably involve a low number of breeders. The low effective population sizes imply a high risk of genetic erosion in domestic stocks.

The main turbot broodstock management programmes are conducted by the two most important turbot producers in Europe: Stolt Sea Farm (Spain) since 1995-96, and France Turbot (France) since 1993. They are both at the third generation of selection for increased growth and the weight gain is around 10-15% per generation. Cryopreservation for turbot sperm is commonly used for broodstock management in commercial hatcheries (24).

Restocking programmes

Experimental stock enhancement exercises (mostly financed by the European Union, national governments and the fishing industry) have been conducted on turbot in Spain, Denmark, Belgium and Norway (5, 9, 25). The efficiency of turbot restocking programmes is often difficult to evaluate as information on natural stocks abundance is rarely or only partially available. Most of these programmes were conducted with juveniles (0+ or 1+ year class), which were released on shallow coastline habitats. Released turbot generally exhibit a relatively high mortality rate, similar to that of the wild turbot (5). Although there are differences in prey and feeding behaviour between wild and hatchery turbots (19), released hatchery turbots seem to be able to adapt to natural diet within a few weeks (25). Releases of hatchery turbot have been proposed to enhance fisheries recruitment, integrated in a resource management programme of sustainable fisheries and habitat restoration.

Interaction studies

There are no studies on genetic interactions between farmed and wild turbots. Althogh no information on escape events is available, escapees are likely to be rare in land-based facilities, especially in those with water recirculation system, and are probably higher in floating cages or in open-circuit pumped seawater. In stock enhancement trials, no information is available on the ability of farmed individuals to survive, mature and migrate to spawning sites. As adaptation of farmed juveniles to the wild is good, it is urgent to investigate those aspects of primary importance to estimate the magnitude and the importance of genetic impacts of farmed turbot on wild populations. Genetic introgression and admixture of wild and domestic genomes is theoretically possible. If transgenic turbot or individuals from monosex female stocks, which are both nowadays in early stages of development, are soon produced, their impact on wild populations must also be investigated. Gynogenetic individuals are able to mature (23). Therefore, the use of triploids to ensure genetic confinment of all genetically modified individuals should soon be explored.

Conclusions/Implications

There are many aspects to be studied for this species before a proper assessment of the genetic impact of aquaculture in wild turbot populations can be performed. Some of the more urgent priorities are:

- Development of new polymorphic markers, necessary to increase the discrimination power of genetic analysis (for genetic identification of different stocks)
- Assessment of the extent of introduction of domestic genomes into wild populations, and its consequences in hybrid generations at different levels (ecological, fitness, etc)
- Assessment of traits ecologically important for turbot adaptation
- Investigation of the genetic architecture of traits associated to local adaptation and domestication

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Blue mussel - *Mytilus edulis* Mediterranean mussel - *M. galloprovincialis*

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Biology, ecology and genetics

Distribution

In Europe there are three species of mussel, all in the genus *Mytilus*: *Mytilus edulis* (blue mussel), *M. galloprovincialis* (Mediterranean mussel) and *M. trossulus* (Baltic mussel) (1, 2). *M. trossulus* is generally thought to be confined to the Baltic (3) and there is no significant fishery or aquaculture involving this species. Because of this, the genetic impact of human activity is negligible and the Baltic mussel will not be considered further in this chapter. On the other hand there is very extensive mariculture of *M. edulis* and *M. galloprovincialis* almost throughout their distribution. There remains debate about the true taxonomic status of these two "species" because wherever their distributions overlap they can hybridise and their hybrids are fertile. Identification of *M. edulis* and *M. galloprovincialis* (and any hybrids) based on shell shape is usually uncertain because of the extreme plasticity of shape exhibited by mussels under environmental variation. Since 1995 a DNA-based genetic method that seems truly diagnostic for European populations of these two species (4, 5) has become available, but no large scale studies have yet been carried out to characterise the mosaic of populations of *M. edulis*, *M. galloprovincialis* and their hybrids that extend from the French Atlantic coast up to northern Scotland. The knowledge of the distribution of mussel species around Europe, as it was assessed in 1992, is given in Fig.1 (1). Hybrids occur in regions where the species meet or overlap. Recent anecdotal evidence suggests that *M. galloprovincialis* could be present in the Netherlands, and that *M. edulis* has been introduced into culture on the Mediterranean coast of France.

Fig. 1. *Approximate distributions of* M. edulis, M. galloprovincialis, *and* M. trossulus *in Europe (1).*

Biology

Mussels are bivalve molluscs and much is known of their biology (1) mainly because they have been an easy marine organism to collect and study. The two valves of the shell are equal in shape and size and are held tightly closed by a large posterior adductor muscle when the mussel is exposed to air. Feeding and respiration are carried out via currents of water directed across the gills. Food particles are trapped by cilia on the gills and carried in mucous strings to the mouth but this is a selective process and some particles are rejected as pseudo-faeces before entering the gut.
The foot of a mussel is an important organ because it enables the mussel to attach by byssus threads to a solid substrate. Threads can be broken and replaced at will allowing mussels to re-orientate themselves within clumps or in rock cracks or crevices. Byssal attachment is an important, sometimes critical, factor in mussel aquaculture.

Maturation of eggs and sperm takes place in the gonad tissue that develops within the folds of the mantle and, depending on environmental temperature and food availability, mussels may spawn just once or several times each year. The sexes are separate in mussels, but there are no morphological differences between males and females. Even in ripe mussels where the mantle is packed with gametes (eggs or sperm) mantle colour is not always a reliable guide to the sex of an individual because eggs can range in colour from white to orange/pink. Two days after fertilisation, embryos develop into planktonic veliger larvae that are dispersed by ocean currents. Larvae settle after 4 to 8 weeks at a size of 250-300 microns shell length (7) and may have a secondary post-larval dispersal phase by "byssus drifting" up to a size of 2mm shell length (8).

Due to their high fecundity, their extensive larval and post-larval dispersal capability, their ability to attach by byssus threads to non-specific substrates and also to one another, and their fast growth, mussels are often a very significant and abundant element of the ecology of many inter-tidal and sub-tidal habitats (1).

As with other bivalves, mussels are affected by "red tides", blooms of certain algae that are poisonous when consumed by humans and induce PSP (Paralytic Shellfish Poison), DSP (Diarrhetic Shellfish Poison) or ASP (Amnesiac Shellfish Poison). Mussels from affected areas cannot be sold and this causes major problems for mussel farming.

Population genetics

The genetics of mussels have been extensively studied. Allozymes were the main genetic markers used from the 1970s to the 1990s and their use culminated in the recognition of, and the world-wide distribution of, the three *Mytilus* species (2). From the early 1990s genetic studies have tended to focus either the hybrid zones between species (9, 10, 11), or the unusual mode of mitochondrial DNA inheritance (Doubly Uniparental Inheritance) discovered in 1994 (12, 13). Although some nuclear DNA markers have been developed for species identification (5, 14, 15) it is only very recently that any microsatellite loci have been isolated for mussels and these have yet to be employed in any extensive population study (16).

The main conclusion from population genetic studies using allozymes was that *M. edulis* was genetically homogeneous throughout its range. On the other hand, *M. galloprovincialis* is genetically subdivided into a Mediterranean group and an Atlantic group with a break point at the well defined Almeria-Oran oceanographic front in the Mediterranean Sea (17).

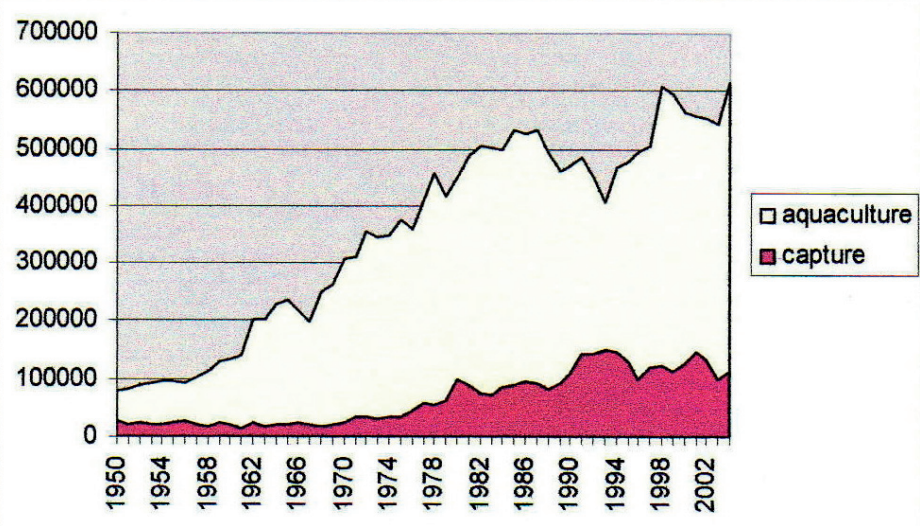
The true extent of the mosaic of population structure across the huge hybrid zone of these two species is not known in detail except at one or two restricted locations (e.g.

10, 11). However, now that a simple and cheap diagnostic DNA-based marker (4, 5) is available, and some microsatellites have been published, a programme of extensive sampling of mussel populations around Europe is warranted.

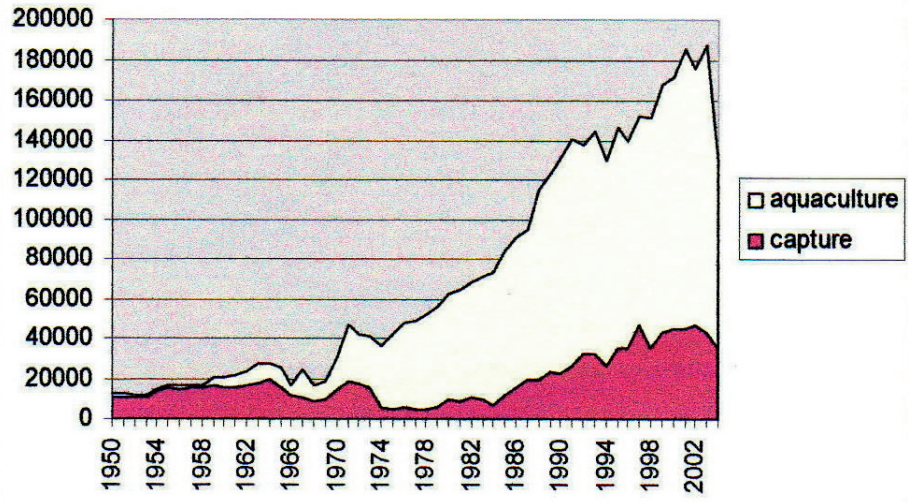
Breeding and culture practices

Production

M. edulis and *M. galloprovincialis* are the most important mussel species in Europe. Total European tonnage of aquaculture and capture from 1950 – 2004 are given in Fig.2. It should be noted that the statistics provided by FAO (6) treat mussels from the Atlantic coast of Spain as *M. edulis*, but, as the distribution map (Fig.1) shows, the species in this region is *M. galloprovincialis*.



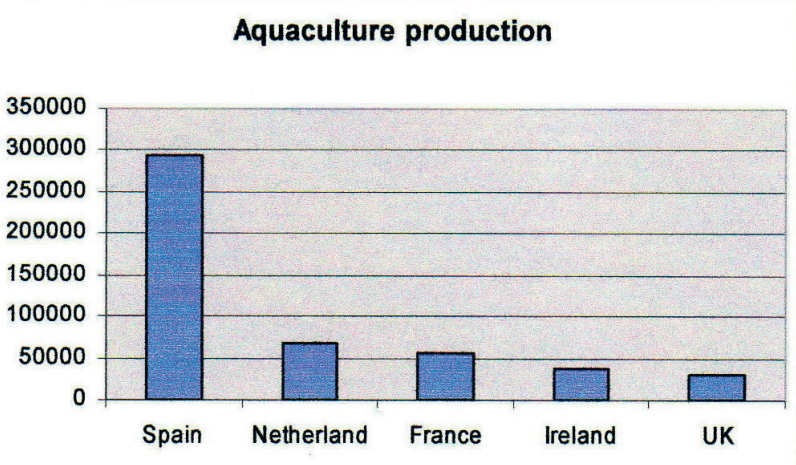
(a) *M. edulis*



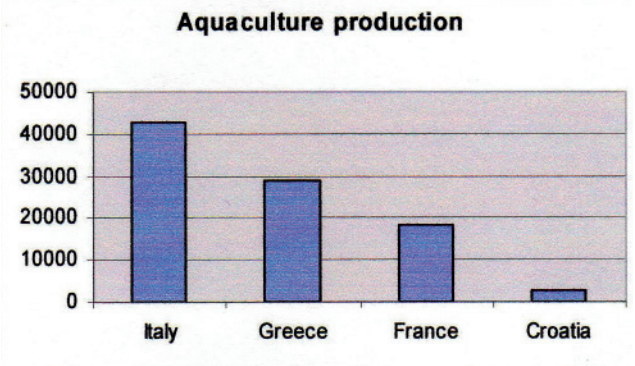
(b) M. galloprovincialis

Fig. 2. *Total mussel tonnage in Europe: aquaculture and capture, 1950-2004 (6).*

European aquaculture production for 2004 is given for main producing countries in Fig.3. Note that Spanish production given as *M. edulis* in Fig. 3(a) should be as *M. galloprovincialis* in Fig. 3(b). Spain is by far the greatest producer of mussels by aquaculture (300 000 tonnes annually), greater than the combined total of other important mussel producing countries such as Netherlands, France, Italy, Ireland and UK (Fig.3). European countries produced 38% of world production in 2003.



(a) *M. edulis*.



(b) *M. galloprovincialis*

Fig. 3. *European aquaculture production of mussels in 2004 from main countries (6).*

Hatchery practices

Because natural spatfall has generally been sufficient to supply most or all of the mussel farmers' requirements in Europe, there has been little interest in developing hatchery culture of mussels. There is at least one commercial hatchery in New Zealand that regularly provides rope culture farmers with hatchery produced mussel spat, but there is no hatchery production of mussels in Europe. However, because of the recent expansion of the industry in northern Europe and fierce competition for variable, possibly dwindling, seed mussel stocks, there is now interest in adapting well-tried oyster hatchery culture methods on mussels (19).

Grow-out

European aquaculture of mussels relies on natural spatfall and there are three main methods of culturing these spat up to market size: bottom cultivation, *bouchot* culture and the suspended rope method (18).

Bottom cultivation involves dredging mussel seed from areas where there is extensive natural annual spatfall and relaying the spat in low inter-tidal or sub-tidal lays in shallow sheltered productive areas. One-year-old seed dredged for relaying ranges in size from 10-30mm shell length. Larger, but slow growing, mussels can also be moved from high inter-tidal areas down to sub-tidal beds where growth rate is increased. This method is most widely used in the Netherlands but has been significantly exploited elsewhere (e.g. the Menai Strait, Irish Sea).

The traditional French bouchot method of mussel culture is carried out on wooden poles that are placed upright into the sea bed in the low inter-tidal region. Natural settlements of mussels are collected on sub-tidal poles or horizontal lines of coir rope during the spring and are transferred onto growing bouchots later in the year. Mussel spat can be mechanically loaded into a long "stocking" of natural fibre that can then be wound around the bouchot. As the stocking fibre rots, the mussels attach by byssus to the bouchot. Synthetic fibre stocking material can also be used because the mussels can force their way out through the mesh as they grow.

Suspended ropes for the culture of mussels can be deployed from large floating rafts or from sub-surface longlines. Raft culture is the commonest in sheltered Spanish rias (estuaries) while sub-surface longlines are used in both sheltered and more exposed sites. Natural settlement of spat is often sufficient to produce commercial quantities of mussels, but spat from elsewhere can also be applied directly to the ropes by using a version of the stocking method where the rope runs down the centre of the stocking. Mussels on bouchots or long lines can be harvested after about 18 – 24 months of growth.

Because these three methods place mussels into rather different environmental situations and predator exposure, it is possible that there may be genotype-specific differential mortality during culture.

Because of the long period of the year when the marketing condition of mussels is poor, due to the fact that they are spawning or spent, there is interest in the potential of sterile triploid mussels that could enable year-round marketing. This has been achieved with Pacific oysters and now most sold in the USA, and many in France, are triploid. Triploid mussels have been produced experimentally in the laboratory (20) and also at pilot commercial scale in Canada (21).

Selective breeding

Although environmental effects are very strong on mussel growth, genetic variation in growth rate has been documented (22). Reasonably high heritabilities have been estimated for larval and juvenile growth in mussels (23, 24) and a good selection response for growth rate achieved (25). These facts suggest that there is considerable capacity for breeding programmes to provide improved strains within both species for particular culture methods.

At present the CAWTHRON Research Centre in New Zealand is running a selective breeding programme and they report positive selection response. In Europe there is a need to develop the necessary hatchery and nursery infrastructure to spawn and rear mussels before any selective breeding programme is possible. However, there seems to be much to be gained in domestication of the mussel.

Interaction studies

The precise distributions of the two commercially important European mussels (*M. edulis* and *M. galloprovincialis*), and the extent of their hybridisation, remain to be characterised for most areas of the European Atlantic coast. Without such basic information the genetic impact of mussel aquaculture, particularly in relation to species interactions, will be extremely difficult to assess.

It is thought that *M. galloprovincialis* is slowly spreading northwards invading territory once exclusive to *M. edulis* and that this may be partly a result of global warming. Whether or not it has been involved so far, it is likely that climate change will increase the rate of this process. It is debatable whether short-term (5-10 year) crisis management of species interactions is worth the effort in the face of longer term (50-100 year) climate change factors.

In areas where aquaculture activity is the major source of mussel biomass it is possible that there may be genetic impact due to genotype-specific mortality related to the particular culture method used.

Hatchery culture of mussels is in its infancy and, in the short to medium term, is unlikely to represent more than a small percentage of the natural spat production of mussels in Europe. For this reason there is negligible risk of genetic impact. However, in the longer term, if domesticated strains of either species are developed and extensively cultured there is certainly the potential for genetic impact.

Triploid mussels, if these prove to be a commercial commodity, could be produced in Europe. However, based on experience with the Pacific oyster and other bivalves, their genetic impact is likely to be low. Triploid bivalves are generally almost sterile and any gametes produced are aneuploid and infertile.

Conclusions/Implications

- It is essential to precisely characterize the true distributions of *M. edulis*, *M. galloprovincialis* and their hybrids in all regions, but especially where mussel aquaculture takes place.
- Based on such a survey, a series of sites should be identified that are to be genetically monitored on a regular basis to identify any changes in species composition over time.
- More microsatellite genetic markers should be developed for mussels to better identify (a) species differences, (b) the processes of hybridization and introgression (c) fine-scale within-species genetic variation and (d) potential genetic variation between natural, rope cultured and bottom cultured mussels
- Phenotypic and genetic parameters for quantitative traits are scarce and should be studied and response to selection for growth and survival should be investigated.
- Triploid mussels should be produced and tested for their potential genetic impact on native diploid stocks.

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European flat oyster - *Ostrea edulis*

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Biology, ecology and genetics

Distribution

The European flat oyster, *Ostrea edulis,* a native of Europe, occurs naturally from Norway to Morocco in the North-Eastern Atlantic and in the whole Mediterranean Basin (Fig.1) (1). It has been a harvested species for at least 6000 years. Natural populations are also observed in eastern North America, from Maine to Rhode Island, following intentional introductions in the 1940s and 1950s. The species was also introduced in Canada for aquaculture purpose 30 years ago and some populations naturalised in Nova Scotia, New Brunswick and British Columbia. These stocks were imported from naturalised populations in Maine whose ancestors originated in the Netherlands (2).

Fig. 1. *Distribution of* O. edulis *(1)*

Biology

Ostrea edulis, whose lower (left) valve is convex and upper (right) valve is flat, lives on firm ground in shallow coastal waters down to a depth of 20 m. The oyster, which is a prominent mollusc in the intertidal zone, like other bivalves, can reach other sea areas in its larval stage. The length of the adult oyster is around 10-12 cm. *O. edulis* can be found in estuaries, and tolerates salinities of up to 23 ‰. It often occurs in large beds on muddy-sand, muddy-gravel and rocks. Oysters filter phytoplankton and other particulate material from the seawater.

O. edulis is a protandric hermaphrodite, changing sex generally twice during a single reproductive season. Oysters function as males early in the spawning season and later change to females before changing to males again. *O. edulis* exists as a series of physiologically different strains, and genetic differentiation has been demonstrated along the European coastline. *O. edulis* produces up to 1 million eggs per spawning that are liberated into the pallial cavity where they are fertilised by externally released sperm. Following an incubation period of 8-10 days, depending on temperature, larvae (160 µm in size) are released into the environment and spend 8 to 10 days as a pelagic dispersal stage before settlement. Appropriate larval growth and survival rates are obtained in 20‰ salinity, although they can survive at salinities as low as 15‰.

Population genetics

The nuclear genetic diversity and geographical structure of *O. edulis* populations has been investigated, mostly by using enzymatic markers (1, 3), but more recently with microsatellite and mitochondrial markers (4, 5, 6). These studies

have revealed moderate differentiation between Atlantic and Mediterranean populations (*Fst* = 0.058 between the two seas). Based on a lower genetic diversity of Atlantic populations, it was considered that these Atlantic stocks originated from Mediterranean populations, after the last quaternary glaciation (1), some clinal and V-shaped patterns of allelic frequencies were interpreted as the result of interglacial secondary contact of Atlantic and Mediterranean stocks (3). The question of the genetic discontinuity between the two basins was thus left open, and has recently been reassessed. A survey based on 5 microsatellite loci (5) has revealed a good correlation between genetic and geographic distances supporting isolation-by-distance as a model and rejecting non-equilibrium scenarios (colonisation or secondary contact). A more recent study (6) compared mitochondrial and nuclear data and showed that the geographically extreme populations sampled in Norway and in the Black Sea appeared particularly differentiated. Furthermore, a clear reduction of female gene flow has been observed and has been interpreted as being a consequence of a biased sex ratio, a higher variance in reproductive success of females and the presence of epizooites. Moreover, the individuals that settled on a collector during two weeks in spring 1994 in a Mediterranean population showed a significantly lower variability in reproductive success than the local adult population (7).

The conditions exist for local adaptation to occur and local patterns of genetic diversity to be observed and analysed. In order to further document this hypothesis, two experiments were conducted at population level. First, brooding females were sampled in the wild and the number of males fertilizing a given female estimated. Then, parentage was analysed for the individuals reared under experimental conditions. Fertilized eggs resulting from successive mass spawnings were collected from a population of potential parents kept in hatchery and with known genotypes. Resulting relative contribution of each progenitor showed a high variance in the reproductive success of males. Furthermore, different patterns of spawning were distinguished: unique, successive or extended in time, providing insight into the reproduction dynamics of this species.

As with other bivalves, heterozygozity deviations from the Hardy-Weinberg equilibrium have been reported in *O. edulis* for allozymes (8, 9) and microsatellites (5). In addition a positive correlation between multi-locus heterozygosity (MLH – both allozymes and microsatellites) and life history traits such as growth or survival has been demonstrated in *O. edulis* (9, 10, 11).

Breeding and culture practices

Production

Ostrea edulis has been part of the human diet for many centuries. The Romans built ponds to stock and sort oysters. In the 17th century, oyster spat were collected on rocks, separated from each other and deployed into ponds in salt marshes on the Atlantic coast of France. A decline in activity in salt marshes facilitated oyster culture development by expanding grow-out acreage availability. During the 18 th and 19 th centuries, fishing effort led to over-exploitation, failing recruitment, and destruction of European natural beds, which were also affected by extremely cold winters. Shortage in seed supply prompted the managers to develop cultural practices aimed to sustain a repletion and reseeding programme. Within the past forty years production of *Ostrea edulis* showed a drastic decline from a peak output of nearly 30 000 tonnes in 1961, due to the impact of two parasitic epizooites (*Bonamia ostreae* and *Marteilia refringens*) in the 1960s (12) and a consequential shift to the rearing of

the Portuguese cupped oyster (*Crassostrea angulata*), then the Pacific cupped oyster (*Crassostrea gigas*).

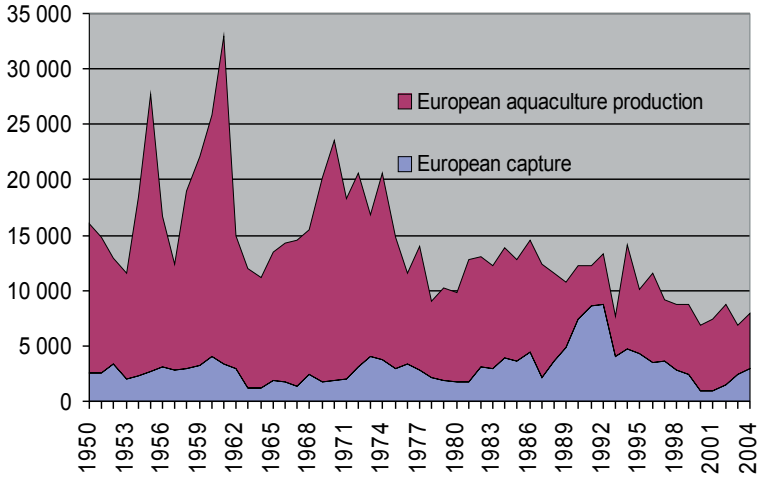

Fig. 2. *Capture fisheries and aquaculture production of* O.edulis *in Europe (in tonnes) (13)*

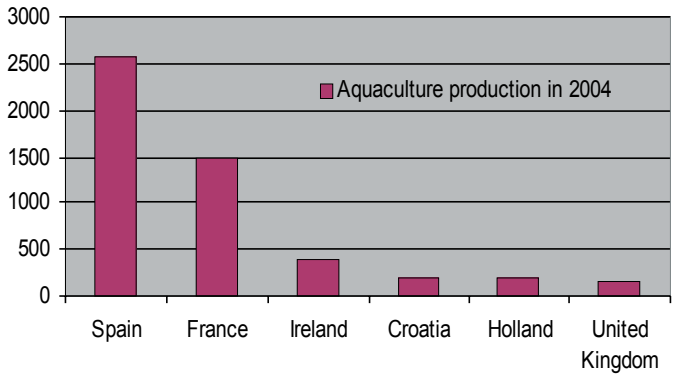

Fig. 3. *Aquaculture production of* O. edulis *in Europe by country (in tonnes) (13)*

The recruitment of natural spat from this species has been strongly reduced and for instance, the French production of flat oyster by a factor of 20 since the 1960s, but is still 1,500 tonnes/year at present. In 2004, 51% of the production was in Spain (2,575 tonnes) and 30% in France (1,500 tonnes). Ireland and Croatia were the other countries that produced more than 200 tonnes in 2004 (Fig. 2, 3). Catches of wild *O. edulis* represents 10 to 30% of the total tonnage of oysters marketed in the recent years. The production of the European flat oyster represented less than 0.11% of the total global production of all farmed oyster species in 2004. The bulk of the world production (96.2%) was the cultured Pacific cupped oyster, *Crassostrea gigas*.

European flat oysters are traditionally consumed fresh and eaten on the half shell. As the available supply has decreased, average prices have dramatically increased: the wholesale average price for *O. edulis* is commonly 3 to 5 times greater than the cheaper Pacific cupped oyster (*C. gigas*). Therefore, the product now occupies a niche market, and is considered as a luxury seafood item - an expensive delicacy for specialised consumers. However, the value of farmed *O. edulis* production in 2004

was US$ 20.3 million. Hence, its culture remains an important industry in the limited areas where it is reared.

Hatchery practices

In most countries, the production of *O. edulis* is still mainly based on wild spat. During the 1960s and 1970s, knowledge about oyster reproduction and rearing techniques improved greatly. However, larval rearing techniques and equipment still rely more on empirical concepts and practice rather than on detailed knowledge of the species biology.

Oysters are alternate hermaphrodites. Synchronous hermaphrodites are rare and selfing is likely to be extremely low. Because flat oysters brood their larvae, strip-spawning is not possible. A single female can release 1-2 million larvae. Under good growing conditions, oysters can produce gametes after a few months such that one-year generation interval is feasible. However, generation interval is usually 2-3 year. In Europe, the main commercial hatcheries are established in France (mostly producing *C. gigas*), the Channel Islands (*C. gigas* and *O. edulis*), U.K. and Ireland.

Selective breeding

The most significant genetic improvement for the production of oysters to date has been obtained through the breeding of triploids. However, in flat oysters, the brooding phase makes the production of polyploids much more difficult and triploid flat oysters are not currently farmed. Quantitative genetics studies suggest that significant gains for disease resistance could be obtained using selective breeding programs. In Europe, where both natural and hatchery-propagated spat are farmed, no large scale selective breeding programmes have yet been started for *O. edulis*. However, several experiments to improve resistance against *B. ostreae*, one of the major parasites and cause of heavy mortality of this species, have been carried out, notably in Ireland (14) and France (15). Results have shown a significant gain in survival and lower prevalence of the parasite in selected stocks. The French experimental breeding programme demonstrated that mass (i.e. individual) selection can improve disease resistance (15). However, the limited extent of hatchery-propagation (*versus* natural recruitment) and/or various technical difficulties and biological characteristics of this species have slowed the development of selective breeding programs. Loss of genetic variability has been documented in mass selected populations (16) indicating that a higher number of breeders should be selected and that the number of progeny tested should be restricted and standardized (17). Also, family-based approaches may be an alternative. Current research includes the development of a genetic map and the search for QTLs of survival to bonamiosis in *O. edulis* (18).

Interaction studies

Compared with fish species, very little is known about interaction between farmed and wild oyster populations because most farmed oysters are neither selected nor domesticated. One of the concerns regarding the genetic impact of farmed oysters on natural populations is about effective population size of hatchery propagated stocks relative to wild populations. This is especially the case for the *O. edulis* in its native area (*i.e.* Europe). Recent studies have suggested that the effective population size of populations might be severely reduced due to much fewer breeding females than males (6). Wild populations may be strongly affected by extensive cultivation of hatchery-propagated spat that is likely to have a low genetic variation. However,

such negative impact remains to be demonstrated. The same kinds of questions are asked for the American oyster, *Crassostrea virginica*, when restoring oyster reefs by hatchery-propagated stocks.

Conclusions/Implications

Data are available on the structuring of the oyster populations in Europe. Although man has been interfering for a long time with flat oyster wild stocks, a low level of genetic structure can still be detected at the European scale. However, the Eastern Mediterranean and Black seas need to be further studied. Here, sampling and efforts are made through the MARBEF Network of Excellence (19). Knowledge of the structuring and the genetic diversity is particularly important in these Eastern Mediterranean countries (Russia, Turkey, Croatia) because some of these are producing or want to develop a production of flat oysters. Research on local adaptation of the populations need to be carried out in order to characterise them as a genetic resource and to estimate the potential impact of domesticated and selected strains.

The effective population size and genetic variation of hatchery propagated stocks relative to wild populations needs to be estimated and recommended broodstock management protocols followed in order to avoid genetic impacts on wild populations.

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Pacific cupped oyster - *Crassostrea gigas*

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Biology, ecology and genetics

Distribution

Originating from the north eastern Asia, *Crassostrea gigas* is endemic to Japan, but has been introduced and translocated, mainly for aquaculture purpose, into several countries, almost worldwide (1). In North America, the species can be found from Southeast Alaska to Baja California, while in European waters the species is cultured from Norway to Portugal as well as in Mediterranean Sea (Fig.1) (2). Biological characteristics make it suitable for a wide range of environmental conditions, although it is usually found in coastal and estuarine areas within its natural range. Although highly variable, the invasiveness pattern of *C. gigas* has been demonstrated in several countries and therefore considered as a pest or a noxious species in those areas (3).

Fig. 1. *Distribution of the Pacific cupped oyster in Europe (2)*

Biology

C. gigas is bivalve mollusc. It is a plankton feeder, filtering phytoplanktonic species for food (filter-feeder) and also ingesting detritic particulate organic matter. *C. gigas* is an oviparous oyster with a high level of fecundity. It changes sex during life, usually spawning first as a male, and subsequently as a female. Spawning is temperature dependent and occurs in summer (15-20°C) synchroneously. Reproductive effort is high, a female producing 20-100 million eggs per spawning (diameter 50-60µm). Fertilisation is external and takes place in the seawater column. At first larvae are free-swimming and planktonic; developing for 2 to 3 weeks before metamorphosis and finding a suitable clean hard substrate to settle on. Highly sensitive to environmental conditions, a very small percentage of larvae survives to become spat. Natural habitat is intertidal and the species can be found down to 15m deep on either hard or soft substrate. The species can resist temporarily to very low salinity (5ppt). The swimming stage and capacity to survive in various environmental conditions facilitate the species dispersion along coastal areas (1).

Population genetics

In Europe, the Pacific oyster was massively introduced after the viral disease that crashed down the Portuguese oyster production by the end of the 1960s. Therefore numerous studies focused on their relationship. *C. gigas* and *C. angulata* had been first classified as two different species based on their apparently separated geographical distribution. However, following morphological comparison,

experimental hybridization (4) and allozyme data (5), some authors concluded that there was only a single species grouping Portuguese and Pacific oysters. Yet, significant phenotypic differences between the two taxa were observed. *C. gigas* shows a superior production yield in the wild in France (6). Differences were also shown in terms of their ecophysiological characteristics (7). Furthermore, genetic differences have been observed at several levels: karyotype analyses (8), mitochondrial (9) and microsatellites (10) studies. In this latter study, a low but significant genetic difference was observed between the French *C. gigas* populations sampled.

Based on these worldwide genetic resources analyses, there might be two putative contact zones, one between France and the south of Portugal where "naturalized" *C. gigas* and *C. angulata* populations have been described, and a second one between Japan and Taiwan. In parallel to the observation of the absence of reproductive barriers under controlled conditions (11), evidence was given for hybridization between *C. angulata* and *C. gigas* in a wild Portuguese population where the two taxa are in contact due to recent transportation of *C. gigas* stocks for aquacultural production (12).

Little is know about genetic adaptation following its introduction into new environments. Results from a common garden experiment comparing progenies of French and Japanese broodstock suggested that the observed differences might be imputable to local adaptation of the French stock since their introduction (6). Polymorphism of presumed selected genes has also been proposed as an alternative method to investigate local adaptation under specific selective pressures such as pollutants (13). Recently, the European Research Training Network on Fisheries-induced Adaptive Changes in Exploited Stocks (FishACE) was set up to investigate the prevalence and consequences of fisheries-induced adaptive changes in French *C. gigas* populations (14).

Breeding and culture practices

Production

Oyster fisheries (*i.e.* exploitation of natural populations as common resource) have, in many cases, shown poor sustainability. Restoration of over-exploited stocks has often been of limited success due to continued exploitation, habitat degradation or diseases. Pacific cupped oyster capture fisheries was never very relevant, with a production of only a few tonnes /year.

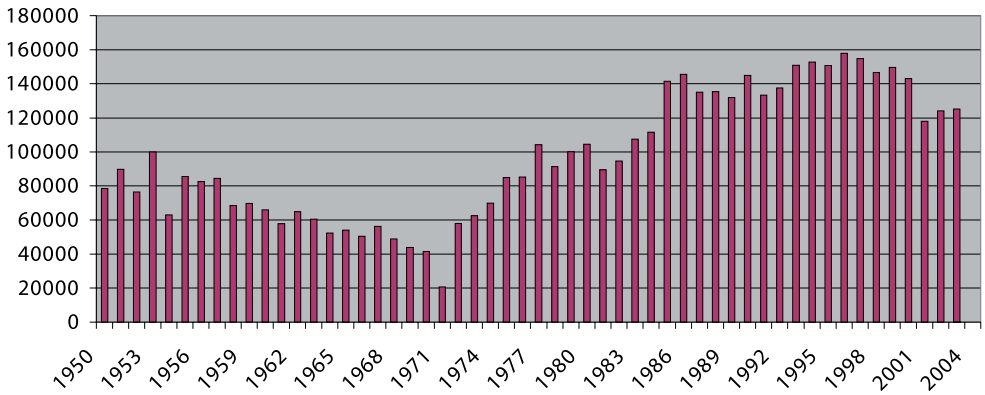

Fig. 2. *Pacific cupped oyster aquaculture production in Europe (15)*

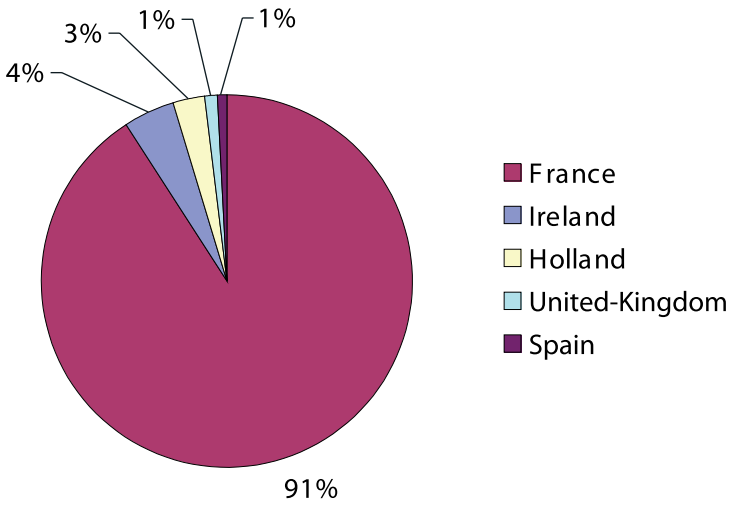

Fig. 3. *Pacific cupped oyster aquaculture production in 2004 in Europe, by main producing countries (15)*

Aquaculture, on the other hand, currently provides most of the marketed oysters and seems to provide a longer term productivity of nearshore marine and estuarine habitats. Farmers grow seed collected from the wild. To date, hatcheries secure availability of seed and allow the production of genetically improved oysters, through polyploidy and selective breeding (see section below). China is the world's leader with 3.75 out of a total production of 4.6 million tonnes in 2004 (i.e. 81% of the world production). European production now ranges around 120 000 tonnes/year (Fig.2), with France, Ireland, Spain, Ireland and U.K as major producers (Fig.3).

Hatchery practices

In most countries, the production is still mainly based on wild captured spat. From the first reported in vitro oyster fertilisation in 1879 to the appearance of modern production hatcheries, hatchery practices have seen more than one hundred years of development (16). During the 1960s and 1970s, knowledge about oyster reproduction and rearing techniques improved greatly. The most recent developments concern the use of high density flow-trough larval systems (as an alternative to batch culture), gamete cryo-preservation and artificial diets.

Today, hatcheries successfully achieve controlled development of spat (immature settled oysters), from fertilisation to post-larvae for many oyster species. Oysters are alternate hermaphrodites. Synchronous hermaphrodites are rare and selfing is likely to be extremely low. In cupped oysters males and female gametes are directly released in the water. Strip-spawning is a common practice in cupped oysters and a fully mature female may yield more than 100 millions eggs. Under good growing conditions, oysters can produce gametes after a few months so that a one-year generation time is feasible. However, generation time is usually 2-3 years.

The proportion of spat produced in hatcheries has increased considerably the last decades, notably in countries were summer water temperature is too low to allow reproduction (e.g. *C. gigas* on the west cost of North America). Additionally, the production of triploid cupped oysters and the establishment of selective breeding programmes enhanced the development of hatchery-produced spat. In Europe, the main commercial hatcheries are established in France, Channel Islands, U.K. and Ireland. In France, most hatchery spat is triploid.

Grow-out

Due to their large worldwide use, oysters are cultured under various rearing strategies and growout equipment from fully extensive to semi-intensive techniques (17). Intensive culture is restricted to early stages (i.e. hatchery and nursery) because large scale production of algal food is not yet cost-effective at later stages. After several months of rearing in open waters, wild oyster spat is either removed from the spat collectors to be deployed onto culture grounds (on bottom) on sticks, or into oyster bags on trestles, baskets, suspensions or stay for pregrowing on the collectors, therefore requiring a thinning out or density decrease. This is done in coastal bays as well as inland using semi closed oyster ponds where seawater fills in by gravity and tide effect. Usually, oyster density-stocking biomass is adapted to local carrying capacity and by adapting mesh size to oyster size to maximize current pattern and food availability, ultimately to reduce the rearing cycle time span. Usually, oysters are sorted, graded and stored in clean water before marketing, to remove mud and grit and operate a slight depuration.

Selective breeding

The most significant genetic improvement for the production of cupped oysters to date (18) has been obtained through the breeding of triploids, especially since the development of tetraploids in the mid 1990s (19). Triploid oysters have a much reduced gametogenesis (but are not fully sterile) and re-allocate part of their resources to growth and survival. To date, about 50% of hatchery-produced *C. gigas* are triploid obtained by crossing diploid females with tetraploid males (i.e. "natural triploid"). Chemically induced triploids have been shown to have lower performance compared with natural triploids (20).

Quantitative genetics studies suggest that significant gains, for disease resistance or for other traits of aquacultural interest, could be obtained using selective breeding programmes. However, the limited extent of hatchery-propagation (*versus* natural recruitment) and/or various technical difficulties and biological characteristics of some species have slowed the development of selective breeding programmes. Mass (i.e. individual) selection have been efficiently used to improve growth (21). To date, family-based selective breeding programmes have been established in U.S.A. (22), Australia (Thoroughbred oysters by Australian Seafood industries) and New Zealand (23), mainly to improve growth, yield and shell shape in *C. gigas*. Interestingly, the use of non additive variance and heterosis in breeding programmes is also being investigated in that species (24). In Europe, where both natural and hatchery-propagated spat are farmed, no large scale selective breeding programmes have yet been started for *C. gigas*.

Marker assisted selection, using microsatellites for mixed families approach or QTLs, is currently being investigated. In *C. gigas*, special attention has been paid in Europe to "summer mortalities", for which the causal factors are still unclear. Results have shown that family-based selective breeding can improve spat survival, with no negative impact on growth. As a high heritability was estimated for spat survival against summer mortality (25) current QTL mapping efforts (26) are likely to be successful. In addition, a micro-array approach is in progress to identify differentially expressed genes between resistant and sensitive lines.

Interaction studies

Compared with fish species, very little is known about interaction between farmed and wild oyster populations. This is mostly because most farmed oysters are not yet domesticated nor selected.

Conclusions/Implications

For the wild European populations of *Crassostrea gigas*, the three main points that need to be considered when dealing with the genetic impact of aquaculture on wild populations are:

- Oyster farming is mainly based on these "wild" populations, with commercial hatcheries producing now about 20% of the spat (mainly triploids). Although this species has been introduced recently, we can consider the populations as "naturalized".
- The two closely related species C. gigas and C. angulata hybridize. Hence a genetic impact has already been observed on one reluctant population of *C. angulata* in Southern Europe where *C. gigas* aquaculture is present. Even if *C. angulata*, the "Portuguese" oyster, has proved to be originated from Asia since at least 400 hundred years, it is now considered as a European species in Southern Europe.
- *C. gigas* is reproducing and settling now in more northern areas and can be considered as invasive.

Therefore, in order to better analyse the genetic impact of aquaculture on oyster populations, research are needed to (a) characterize the invasiveness of *C. gigas* in Europe [as it is becoming to be done in some countries (27)], (b) investigate the introgression from *C. gigas* to *C. angulata* in the aquaculture areas of southern Europe, (c) investigate at the European level the genetic differences between populations and possible local adaptation.

One of the concerns regarding the genetic impact of farmed oysters on natural populations is about effective population size of hatchery propagated stocks relative to wild populations. Putative negative impact of farming triploid oysters (in Europe: *C. gigas*; in USA: *C. ariakensis*, *C. virginica* and *C. gigas*; in Australia: *S. commersialis*) is related to their partial sterility. Triploidy is not considered as a safe genetic confinement tool as triploids can effectively breed. The impact of this partial sterility on wild populations is poorly known and needs to be investigated. Another risk may come from tetraploid broodstock that are fully fertile. The fate of tetraploid in the wild (*i.e.* their fitness relative to diploids and the impact of their breeding with diploids) is of concern in Europe but needs to be investigated.

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Scallops - *Pecten maximus* and *P. jacobaeus*

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Biology, ecology and genetics

Distribution

P. maximus is distributed from northern Norway down to North Africa (Fig.1 – inset). Extensive fisheries exist for this species around the coasts of France and UK (Fig.1). *P. jacobaeus* is present within the Mediterranean and the Adriatic Sea and has been extensively exploited by local fisheries. The distributions of the two species are not thought to overlap at the entrance to the Mediterranean (1).

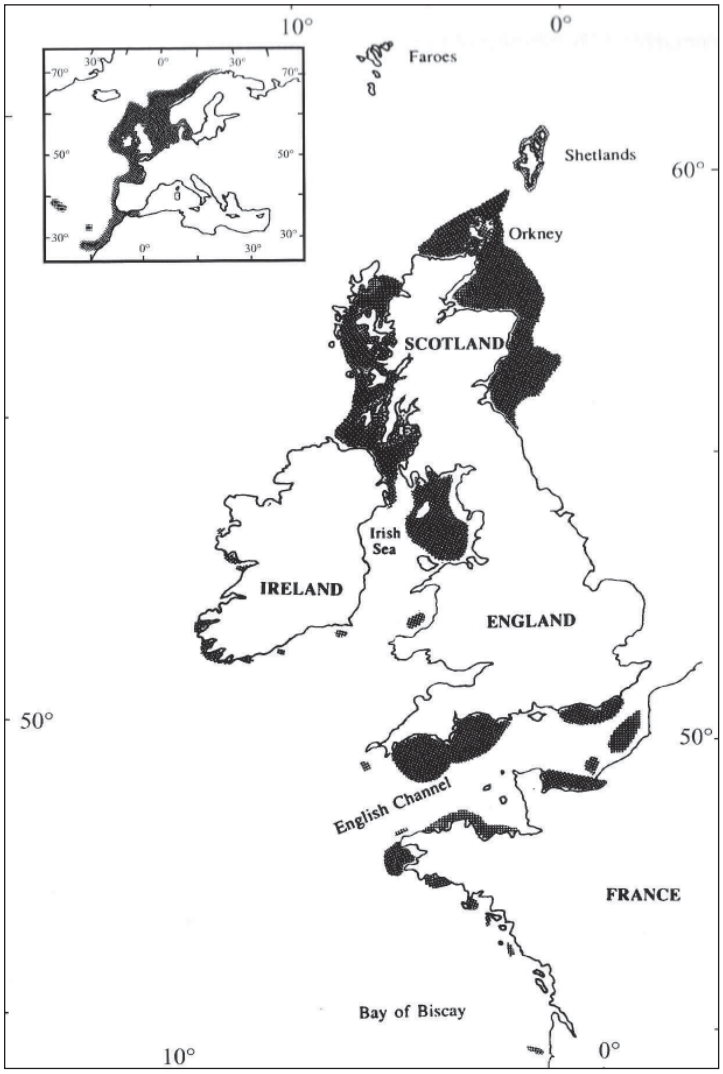

Fig. 1. *Main fishing grounds for* P. maximus *around the UK, Ireland and France. Inset shows range of the species - from (1)*

Capture

Significant dredge fishery for scallops in Europe began in the 1950s around the coasts of UK and France and tonnages are given in Fig.2, with landings by country in Fig.3.

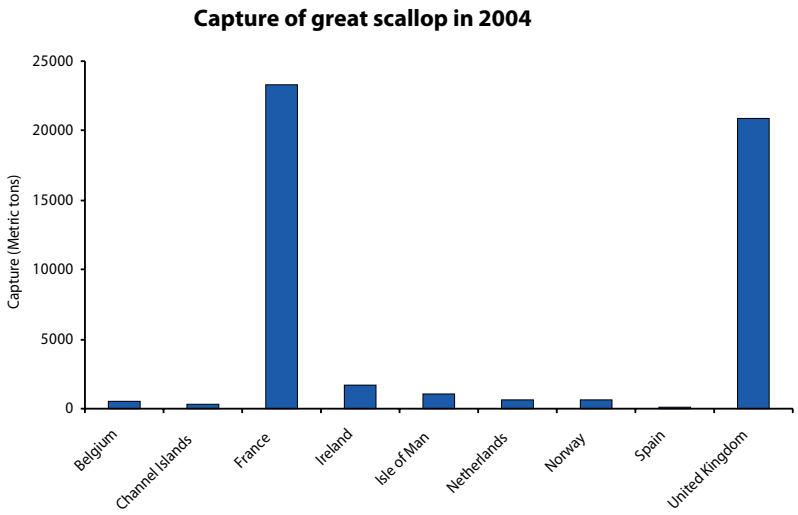

Fig. 2. *Landings of capture fishery of* P. maximus *in Europe, 1950-2004 (2)*

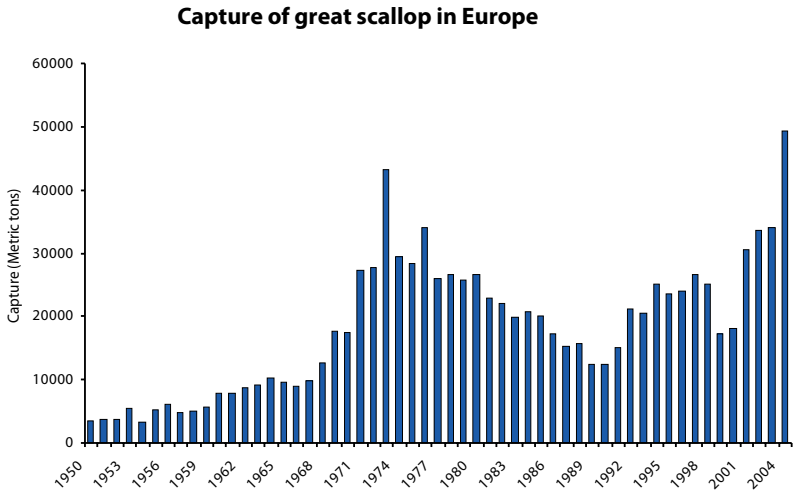

Fig. 3. *Landings of capture fishery of* P. maximus *in European countries during 2004 (2).*

Biology

Pecten spp. are bivalve molluscs in the family Pectinidae. They are filter feeders extracting particulate matter from the surrounding water via a feeding current drawn across the gills by cilia. Food material is trapped on the gills and carried to the mouth in mucous streams. The gills also act as the respiratory organ for the scallop. The shell is secreted by the mantle as the scallop grows. The upper (left) valve is flat and usually reddish brown while the lower (right) valve is convex and paler cream or brown in colour. Both valves can be marked with spots or zigzags of red, pink or yellow. There are prominent ears occupying about half the width of the

shell. There is a sculpture of 12-14 broad radiating ribs on the upper valve (slightly differently shaped in the two *Pecten* species) and concentric annual growth rings are often visible. It takes *P. maximus* up to 4 years to grow to 10-11 cm, the Minimum Landing Size (MLS) for this species in Britain and Ireland (3).

P. maximus inhabits sand or gravel substrates from low water down to 100m depth (3). Growth rate can be affected by several factors including salinity, temperature, competition, water depth and food supply. Growth slows in older individuals and growth rings become closer together and more difficult to distinguish (3).

P. maximus can swim by rapidly clapping the valves and expelling the water on either side of the dorsal hinge and such adductions are also used to excavate the depression within which the scallop sits. Numerous small eyes are present around the mantle edge that are responsive to light (4). Scallops can accumulate poisons during toxic phytoplankton blooms and therefore can induce illnesses such as Paralytic Shellfish Poisoning (PSP) in humans (5).

P. maximus is a hermaphroditic species with a separate tongue-shaped orange / red (female) and white (male) gonad. In northern populations spawning is a single annual event, but several peaks of spawning can occur in southern populations. The trigger for spawning in the wild is not known with certainty but there may be an element of lunar periodicity (1). Laboratory observations indicate that male gametes are usually expelled first and there is a short rest period before eggs are released. Larvae are planktonic for 3-8 weeks and, after initial settlement, post-larvae can become further dispersed by byssus drifting (6). Details of the processes involved between initial spat settlement on filamentous substrates and subsequent recruitment as juveniles onto adult scallop beds remain elusive. The dispersal of larvae and spat is influenced by factors such as local hydrography, suitability of substrates and the longevity and survival of larvae. Consequently, *P. maximus* exhibits an aggregated distribution with major fishing grounds quite widely separated allowing environmental conditions to produce noticeable differences in population parameters (1).

Population genetics

Two allozyme studies of *P. maximus* (7, 8) revealed very little stock structure within the UK or France. A mitochondrial DNA marker in *P. maximus* (*Pma1*) was developed which also failed to reveal any significant population structure except at one site, Mulroy Bay in Ireland, which differed significantly from all other sites (9). Other mtDNA and RAPD markers studies (10, 11) also showed the Mulroy Bay population clustering separately from others. Norwegian *Pma1* haplotype frequencies are rather different to those in UK sites, but no differentiation has been identified between Norwegian populations (12).

There is therefore little evidence of substantial genetic differentiation of *P. maximus* populations throughout its range apart from Mulroy Bay. Mulroy Bay has been a regular source of scallop spat for aquaculture enterprises elsewhere in Ireland (13) but scallop recruitment may now be suffering due to extensive mussel rope culture in the bay.

As with other bivalves, heterozygote deficiencies have been reported at a number of loci in *P. maximus* and the most likely causes are selection and/or the presence of null alleles at the scored allozyme loci (14). A significant positive relationship between allozyme polymorphisms and growth or other fitness parameters, the so-called "Heterozygosity-Fitness Correlation" (HFC), has been demonstrated in many bivalves but studies on scallops have generally failed to find a significant HFC (14).

Taxonomic relationship between P. maximus and P. jacobaeus

Although there are morphological distinctions between *P. maximus* and *P. jacobaeus*, various genetic markers have failed to identify deep genetic separation (15, 16, 17, 18). The most recent study using mtDNA suggests that the two species shared a common ancestor fairly recently in the Pleistocene (19).

Breeding and culture practices

Production

Scallop culture started late in Europe compared with other species of molluscs. In 1984 the total aquaculture production of scallop was 78 600 tonnes of which 94% came from Japan. Recently China has surpassed Japan and in 2003 China alone produced 76% of the world aquaculture production of 1.17 million tonnes. In Europe, Spain, France, Ireland, UK and Norway have been producers of scallop and the aquaculture production reached a peak in 1998 with 512 tonnes followed by a reduction to 213 tonnes in 2004 (Tab.1). The estimated value of the European production in 2004 was 852 000 € (4€/kg).

Country	Highest production in 1990s	Production in 2004
Spain	207	0
UK	188	64
France	150	0
Norway	132	46
Ireland	67	103

Tab. 1. *Aquaculture production of* Pecten maximus *in Europe, tonnes (2, 20)*

Hatchery practices

The production of *P. maximus* is still mainly based on wild captured spat. However, the proportion of spat produced in hatcheries has increased over recent decades. Adult scallops can be conditioned in water enriched with microalgae and can be induced to spawn as the water temperature is increased. Larval culture of scallops is essentially similar to the well tried method used commercially for oysters. After 3-4 weeks, spat are collected on a settlement substrate and are later moved to nursery tanks or put into trays and cultured on sea-based longlines.

Grow-out

After about 2 months the spat should reach about 10 mm and may be on-grown by a variety of methods such as (a) hanging culture using lantern nets, pearl nets, or ear hanging, (b) bottom culture using a fenced area on the sea bed and (c) bottom ranching, putting large scallop spat directly onto the sea bed in unprotected areas with few predators. The most serious predators for scallops are starfish and crabs, but also fish (Ballan wrasse, *Labrus bergylta*) prey on juvenile scallops (21).

There are several possibilities for scallops to escape from the hatchery, from longlines and in particular from sea ranching activities. Since domestication is at an early phase, the effect on wild populations is probably not significant, but hatchery-reared scallops have been extensively re-seeded into bays in northern France.

Selective breeding

Few quantitative genetic studies have been performed on scallops. In Japan, broodstock of *Patinopecten yessoenses* were collected from the wild, and later they were selected from farmed specimens, but it is not known how selection was practised, which traits were considered, nor the intensity of selection. Some selfing possibly occurs in the wild but it is very difficult to avoid it completely in hatchery activities. Selfed larvae exhibit significantly reduced growth in *P. maximus* (22) and lower growth and survival in the Mexican scallop, *Argopecten ventricosus* (23).

A cross between two populations of *A. ventricosus* tested in two different environments did not show "useful heterosis" for growth or survival in either environment (24). However, the genotype – environment interaction was significant for four growth traits and for survival. Selection experiments (breeding from the best 10%) have demonstrated a 16% gain in weight per generation (25) with a realized heritability for weight of 0.33 ± 0.08 to 0.59 ± 0.13 and for shell width of 0.10 ± 0.07 to 0.18 ±0.08. A correlated response in adductor muscle weight, the most important trait in scallop, was predicted to give up to 19 % per generation increase when selecting for total shell weight (25).

Heritabilities ranging from 0.21 to 0.37 have been reported for growth rate in the American bay scallop, *Argopecten irradians irradians,* (26, 27) and a 9% per generation genetic gain has been achieved (28). The results from these studies on *A. irradians irradians* and *A. ventricosus* indicate large genetic variation in scallops which is encouraging for *P. maximus* aquaculture and is in agreement with conclusions elsewhere (29).

There is no breeding programme for *P. maximus* but in 2002 a breeding programme for *Argopecten purpuratus* was started in Chile by the Chilean scallop producers Association (30).

There is good evidence that induction of triploidy in scallops could be used to increase muscle growth relative to diploids at market size (31, 32).

Interaction studies

At present there has probably been very little, if any, genetic impact of aquaculture on wild populations of *P. maximus* due to the low level of aquaculture activity. The one exception may be the French re-seeding programme.

It is important to investigate the population substructure of *P. maximus* in order to be able to estimate any genetic impact of aquaculture activity. Some substructure has been identified but much may remain hidden. There are possible inbreeding effects in scallop aquaculture product due to unintentional selfing. This would add to the normal reduction in effective genetic population size when using hatchery broodstock.

Conclusions / Implications

– Efforts should be made using a suite of molecular markers to establish the population structure of *P. maximus* across its whole range, with special reference to localised adapted populations at the extremes of its distribution.

– Breeding experiments should be carried out to investigate quantitative genetic parameters of commercial importance. This is a prerequisite for the development of efficient breeding programmes for the enhancement of *P. maximus* aquaculture.

– Selection experiments should be carried out to study possible genetic gain in fitness traits.

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European lobster - *Homarus gammarus*

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Biology, ecology and genetics

Distribution and capture

The European lobster, *Homarus gammarus,* has a broad geographical distribution (Fig.1). In its northern range, it occurs from the Lofoten Islands in Northern Norway to south-eastern Sweden and Denmark, but is absent in the Baltic Sea probably due to lowered salinity and temperature extremes. Its distribution southwards extends along the mainland European coast around Britain and Ireland, to a southern limit of about 30° north latitude on the Atlantic coast of Morocco. The species also extends, though less abundantly, throughout the coastal and island areas of the Mediterranean Sea and has been reported from the westernmost end of the Black Sea in the Straits of Bosporus (1, 2).

Fig. 1. *Geographical distribution of* H. gammarus

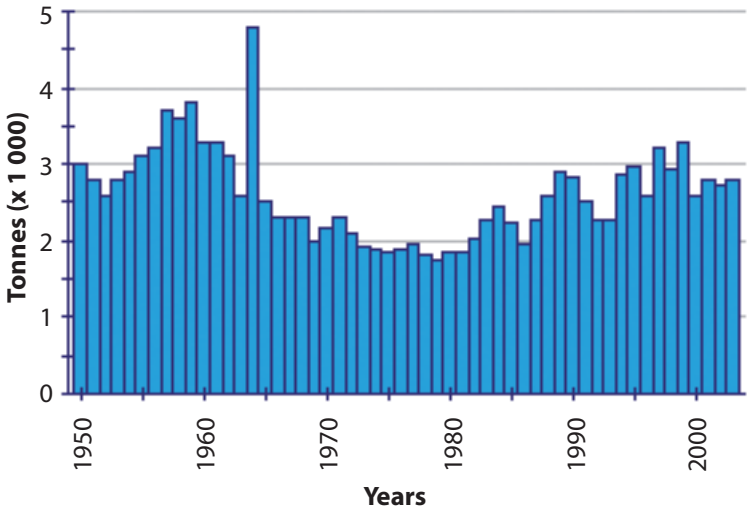

Fig. 2. *Total European capture production for* H. gammarus *(3)*

Capture

Within the past 70 years, total annual European landings have varied between 1 600 and 4 800 tonnes (Fig. 2). In the early 1960s, annual catches topping 3 000 -3 500 tonnes were not uncommon, but landings decreased during the 1970s to below 2 000 tonnes in the early 1980's. Since then, a slow increase to 3 200 tonnes has been observed. Lobster catches vary considerably between countries: between 1950 and 1975, Scotland accounted for 26% of total landings; Norway for 18%, followed by England, Wales and France with 16%, Ireland with 9% and Sweden, Denmark and Spain with less than 5% (4). Prior to the 1960s, Norway recorded annual catches ranging from 600 to 1 000 tonnes, but during the subsequent two decades a collapse in the fishery was observed and annual catches are now less than 30 tonnes. Within the Mediterranean countries annual reported landings have never reached the same levels as those seen in the northern distribution range (5).

European lobster fisheries have so far been either unregulated, or only lightly regulated by national minimum legal size, supported in some countries by national or local prohibitions on landing egg-bearing females and/or closed seasons. However, these have become more comprehensive in recent years to include V-notch schemes for berried females and nursery areas. From January 1st, 2002, a new EU minimum legal size of 87mm CL came into force which is broadly equivalent to the mean size of first maturity but this varies from area to area throughout the range (6).

Biology

European lobsters are usually located at lower than mean low water neaps (sublittoral fringe) to depths of 150m (2, 7). They are primarily nocturnal animals feeding on blue mussels, hermit crabs and polychaetes. Growth is by moult, which decreases in frequency during the juvenile stages until becoming an annual part of the mating, spawning and egg hatching cycle (8). Females can spawn annually or following a bi-annual pattern. Reproduction takes place during summer and is linked with the moulting cycle (9). After extrusion, the eggs are held on the pleopods for approximately another year until hatching the following summer. Large females (>120 mm carapace length) have been shown to moult and then undergo two successive spawns before moulting again, suggesting the capacity for sperm storage (10).

The first few post-hatching weeks are characterised by a pelagic phase usually lasting 14–20 days depending on the water temperature. During this period, larvae undergo four developmental stages until metamorphosis to stage IV (meta-larvae) when they settle to the seabed (11, 12). Despite significant and widespread investigations (13), no information is currently available on the early benthic phase (EBP) of the European lobster from settlement at 5-7mm CL until 20mm+ and juveniles are scarce up to 40-45mm CL. Thus, unlike what is common practice for the American lobster (*H. americanus*), it is not feasible to use EBP or early juveniles to predict future recruitment in *H. gammarus*.

In most areas lobsters do not mature before 5–8 years (depending on water temperature). Genetic data suggest that females in the wild mate with a single male (5). Results from tank experiments demonstrate that individual males can fertilise several females in the same season and this is likely to be the case in the wild. Thus the normal breeding system in the wild is likely to be polygynous (5). In the absence of exploitation the life span is probably in decade. Males reach sexual maturity earlier than females. European lobsters are sedentary animals with home ranges varying from 2 to 10km (14, 15, 16).

Population genetics

As result of the GEL-FAIR (Genetics of the European Lobster) project, the population genetic structure of the European lobster is now better understood in comparison to other marine species. Using a combination of molecular markers (microsatellites, mitochondrial DNA and allozymes) and a comprehensive sampling design involving over 5 000 individuals from 46 locations covering the whole distribution range of the species in Europe, researchers involved in the GEL project reported on an overall low level of genetic differentiation among population samples (5, 17, 18). There was no major evidence for great genetic discontinuities between the Atlantic and the Mediterranean populations in contrast to what has been demonstrated for some other marine organisms. All molecular markers corroborate the existence of four major distinct groups: northern Norway (19); Netherlands; remaining Atlantic samples; and the Mediterranean, in particular the Aegean (17). The northern Norway, Netherlands and Aegean groups differentiate from the main Atlantic group due to reduced gene diversity. Within the major Atlantic group, no correlation was found between geographic and genetic distance. Overall, results from the GEL project indicate that the European lobster is comprised of a large number of discrete populations with limited gene interchange among them (i.e. following an island model of population structure). Although the overall level of genetic differentiation among European lobster populations is low, this does not mean that there are not important adaptive genetic differences present. Indeed, it is extremely likely that lobsters living at the edges of environmental tolerance for the species are adapted to some degree to these conditions. Certainly life cycle parameters are very different in northern Norway and the Aegean (5, 17, 19).

Breeding and culture practices

Production

Lobster aquaculture production, although small, is growing. This trend is being driven by both a noticeable decline in fisheries in parts of the range, and an increased worldwide market demand for lobsters, with *H. gammarus* topping the list as one of the most desirable species.

Hatchery practices

In comparison to other lobster species, *Homarus* species are characterised by a simple and abbreviated larval period. They readily feed on natural and artificial food, are resistant to diseases and exhibit rapid and accelerated growth in warmed waters (20). Temperature is the primary controller of growth, with optimum water temperature around 20-22°C (21, 22). Larval period in 20°C water is around 12 days (23) in comparison to 35 days at 15°C (22). *H. gammarus* can reach 250-300g (total length 210mm, carapace length 75mm) in 24-30 months at 20°C constant water temperature (24). The main factors influencing growth rate in lobsters include handling, stocking density, habitat size, social interactions and water quality (21). Due to the considerable variation in individual growth rate and high losses due to cannibalism and associated injuries when kept in a communal system, cultured lobsters often have to be maintained in separate containers.

Fig. 3. *Juvenile lobsters at the Norwegian Lobster Farm at Kvitsøy, Norway (photo by E. Farestveit).*

Grow-out and restocking programmes

Lobster aquaculture can be carried out in three distinct forms: *product enhancement*, *resource enhancement* and *full grow out*. The latter two practices have been the focus of intensive research over the past 15 years. In *product enhancement*, wild caught lobsters are maintained in pounds where they are fed to improve quality/size (23). In r*esource enhancement aquaculture*, lobster hatcheries are built aiming at hatching eggs, and releasing stage I or stage IV larvae to supplement wild stocks (25). Magnetic binary-coded tags (microtags) and, more recently, genetic tagging allow for the quantitative evaluation of lobster release programmes (5, 26, 27, 28, 29). *Full grow-out*, or close cycle culture, is carried out independently of fishery and involves rearing lobsters from egg to marked size. Until recently, full grow-out culture of lobsters was not considered economically viable given the logistical implications related to the need to keep individual lobsters in separate compartments due to the cannibalistic behaviour and the lack of automated procedures for feeding and maintenance. These problems, however, have been recently addressed. A successful and comprehensive research project focusing on the development of methods for intensive farming of *H. gammarus* in closed system was recently reported (20). In optimal rearing conditions, it is possible to rear a portion size lobster from hatching in 800-900 days (30).

Although intensive culture does increase the likelihood for disease outbreaks, with over a century of experimental and commercial lobster hatchery operations, only few incidents of disease have been recorded (20, 31). Among the causes contributing to disease outbreaks are: excessively high temperatures, possible physiological stressors, poor water quality and inadequate nutrition (23). Disease is best avoided in aquaculture systems through preventive action (e.g. broodstock should be quarantined before being introduced into the hatchery) and a rigorous control over the key water quality parameters (20).

Interaction studies

During the last decades, there has been increasing awareness that aquaculture activities, including stock enhancement and commercial ranching, may have negative impacts on native gene pools. Genetic problems connected to hatchery operations (32) have been discussed in detail, and several recommendations are available (33). The main aim of domestication and selective breeding is to develop high performance strains under farming conditions. This unavoidably results in genetic changes in domesticated stocks. The main genetic concern is that interbreeding between wild and escaped farmed individuals or deliberate releases in enhancement/ranching could result in genetic changes in the wild populations resulting in reduced overall fitness and productivity (7).

In addition to commercial movements between countries, culture of lobster during the early high-mortality stages and then release in the wild has been widely practised as a means of potential enhancement of lobster stocks (29). However, in many cases marking of released individuals has not been carried out to determine the efficacy of the procedure. Where coded-wire or other physical tagging has taken place, this has been limited by the need to rear larvae to sufficient size and by cost, tag loss, etc (29). Furthermore, no account has been taken of the potential genetic changes in native stocks as a result of use of non-native lobsters in ranching or of commercial movements.

Genetic tagging is a viable and powerful approach to address these questions. Until recently, no adequate tools were available for genetic studies in *H. gammarus*. However, microsatellite and mtDNA markers that allow for high resolution genetic studies in this species, including genetic tagging, have now been reported (5). More recently, the genetic fitness of larvae from wild and ranched families was assessed using microsatellite DNA profiling (18). The authors found that the offspring of cultured females displayed relative fitness of 60% in relation to those of wild individuals clearly demonstrating the potential problems of cultured individuals at least during early larval stages (34). It is clear that additional research in this area is required.

European lobster management should be based on local populations i.e. self-recruiting stocks rather than on broader metapopulations as recently favoured by fisheries ecologists. Delimitation of a local stock is not straightforward and is likely to vary throughout the range. Assessment needs to be based on a combination of biological, hydrographical and genetic information. In many European countries wild lobster stocks are at very low levels. Given the information currently available, it would be wise to apply the precautionary principle to movements of lobsters for enhancement purposes. Transplantation of lobster stocks over larger distances should be avoided until much more detailed information on fitness related differences is available. However, the low level of gene flow suggests that lobster culture can be carried out in areas where there are no native populations without adverse impact on adjacent native stocks (5).

Conclusions/Implications

It is of crucial importance to investigate what possible effects domesticated stocks will have on wild populations, particularly for survival and other fitness traits. Furthermore, breeding experiments and genetic studies should be given high priority to increase our knowledge about quantitative genetics in European lobster.

Incidentally, the European lobster is an ideal model species for studying local adaptation. It occurs in a wide range of environmental conditions and produces large numbers of offspring. Since it is relatively easy to transport living females with attached fertilised eggs, it is possible to examine survival and other fitness traits of individuals from two populations under reciprocal environmental conditions. Such movement is at best extremely difficult for fish and many other marine organisms (5).

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I

Genetics of domestication, breeding and enhancement of performance of fish and shellfish

I.2 *Performance improvements by polyploidisation, gene transfer and DNA vaccination in aquaculture*

The following four chapters report the outcomes of the discussions held during the last three sessions of the workshop "Genetics of domestication, breeding and enhancement of performance of fish and shellfish", for which a format based on preordained sets of questions to be discussed and possibly answered to was adopted. Sensitive issues related to the application of technologies for triploidy induction, gene transfer and DNA vaccination in fish and/or shellfish cultures were addressed and responses by chairpersons and other participants summarized as brief statements in order to convey the essential information related to technique applicability, food product safety, and environmental compatibility and containment of the manipulated species. A fourth chapter with general considerations about genetic modified organisms is also included.

Performance improvements by polyploidization in aquaculture

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Introduction

Polyploids can be defined as organisms with one or more additional chromosome sets with respect to the number most frequently found in nature for a given species. For several species, including both wild and farmed ones, some spontaneous polyploids are occasionally found in nature. Also, many plants used in modern agriculture are induced polyploids.

Polyploidy, particularly triploidy, can be easily induced in some invertebrates and lower vertebrates, resulting in viable animals. Regarding species cultured in Europe, polyploidy is currently induced in oysters to improve some aspects of production. According to the EU regulations (Directive 90/220/CEE of April 23 of 1990), polyploids are not considered genetically modified organisms (GMOs).

Since triploids are sterile, their use in aquaculture and restocking has been promoted by several international organizations (NASCO, FAO, ICES, etc.) in order to limit the genetic impact of escapees on wild populations. In addition, their use has been proposed as a solution to the problem of the containment of GMOs.

Summary of responses to questions

1) Effectiveness of current direct triploidization techniques

a) Are there some species that exhibit more intrinsic variations to direct triploidization treatments?

Most of cultured species can be readily triploidized, except some having an unusual reproductive strategy, such as the flat oyster and the lobster. At least in fish, species having large eggs appear to exhibit a variation of response to treatment induction with temperature shocks. Pressure treatment or crossing tetraploids with diploids seems to give more reliable results in these cases, depending on species. Cold shocks and pressure shocks are suitable for fish species with small eggs (carps, seabass, turbot, seabream, etc.).

b) What is the relevance of egg quality for successful triploidization?

For triploidization, egg quality is important to optimize production and have the best possible survival. In batch with poor quality, eggs may display different levels of promptness for the resumption of the second meiotic division with consequent decrement in the effectiveness of the triploidization treatment.

c) Importance of fine-tuned variables to maximize induced triploidy.

Depending on the objective pursued, it is more or less important to achieve a 100% triploidization rate. In order to obtain 100% triploidy, application of a precise protocol is required, including the fine-tuning of triploidization variables (timing, intensity and duration of treatment, as the most important ones). Such protocols

exist for many species farmed in Europe (*e.g.*, seabass, gilthead sea bream, turbot, trout, oyster), but need to be optimized for others.

In the case of GMOs farmed in the open environment, 100% sterilty is critical and needs to be achieved and confirmed. For non-GMOs, the higher the level of sterility achieved the better in order to limit interaction with the wild population.

d) Importance of an adequate scaling-up of the method.

An adequate scaling-up of the method is a key step if triploidization is to be applied at the large scale required for production and should be developed in collaboration with the aquaculture industry.

e) When treatment induces lower larval performances (especially survival), what level of triploidy- or treatment-induced depression can producers accept?

In some species, triploid induction implies some mortality at the early stages of development, before investment in food and resources has been made. This mortality has to be balanced with the expected ecological and economical benefits derived from the application of triploidy.

2) Performance capacity of triploids with respect to diploids

a) What is the performance of triploids in culture as compared to diploids?

Performance of triploids is species specific. Currently, triploids are farmed to maintain or improve organoleptic quality when the product is sold during or after sexual maturation of diploids (oyster, salmonids). In some cases, triploidy improves growth and survival (oysters) but, particularly in fish, it usually does not. However, it avoids growth depression and the increase of mortality associated with the sexual maturation of diploids, resulting in increased production with triploids (*e.g.*, salmonids, turbot). Under good culture conditions, triploids perform well, but under adverse conditions (e.g., low oxygen concentration, stress, etc.), they may underperform relative to diploids. There is insufficient evidence to indicate that triploids are more prone to developmental abnormalities.

b) What information is available on the performance of triploids in the wild and do they show any sexual behaviour?

Apart from the lack of homing behaviour of sterile Pacific salmon, little is known about the performance of induced or natural triploids in the wild. Male triploid Pacific salmon display sexual behaviour in the wild and could interfere with reproduction of native individuals. There are no data on European species.

3) Degree and permanence of gonadal sterility in triploids

a) Does triploidization produce functional sterility in both sexes?

Triploidy interferes with gametogenesis in all European aquacultured species tested so far. In fish, it results in complete female sterility, but males are still able to develop testis. In some species (*e.g.*, Atlantic salmon), these males can produce only a small amount of aneuploid sperm, capable of activating eggs, but not fertilizing them. In other species (seabass, turbot, seabream, Arctic charr), triploid males do not produce sperm. In the Pacific oyster, some triploids can develop male and female gonads. Often male oysters release sperm and, exceptionally, females can release eggs.

b) How many reproductive seasons do we have to monitor before ensuring that triploidy induces functional sterility in a given species?

To ensure that triploidy induces functional sterility in a given species, at least two full consecutive reproductive cycles should be monitored and the absence of gamete production confirmed.

c) Are current techniques for triploidy induction adequate for the biocontainment of GMOs?

For GMOs, where 100% biocontainment is required, the current technology based on the production of triploids cannot ensure it, unless a feasible method for the large scale monitoring of ploidy on an individual fish-by-fish basis is developed. Complementary technologies (physical containment, sterile hybrids, anti-sense GnRH transgenics, etc.) should be considered.

4) Applicability of tetraploidy to generate auto- and allotriploids

a) What are the effects on viability and reproductive performance of tetraploids?

Tetraploidization is theoretically possible, but in practice difficult to achieve. Viable tetraploids have only been produced in Pacific oyster and rainbow trout, and are fertile. In Pacific oysters, crossing tetraploids with diploids gives 100% triploid stocks. In this case, tetraploids are appropriately contained to avoid larvae or eggs escaping into the environment. In trout, production of unreduced eggs and enlarged spermatozoa in tetraploids limits their value as a tool to produce triploids.

Viable tetraploids were not possible to obtain in sea bass or carp. In other species it has not been tested and thus valid conclusions cannot be drawn.

Main research priorities for the potential use of triploidy to limit the genetic impact of escapees on wild populations

1. If sterility or biocontainment is found to be necessary for species currently produced and for which commercial triploidization protocols are not available (cod, meagre, soles, sturgeons, mussels, pectinids, abalone, etc.), these should be developed.
2. In spite of the success of tetraploidization in oysters, tetraploid fish are difficult to produce. Further research is required in this area.
3. Knowledge on the physiology and gene regulation in triploids (hormonal and immune status, functional genomics) reared in optimal or sub-optimal farming conditions should be increased.
4. The mechanisms by which triploidy affects reproduction, particularly gametogenesis, are still poorly understood and should be investigated by using a range of experimental approaches.
5. Performance of triploids under both normal and adverse conditions (stress, lower water oxygen concentration, presence of pathogens) needs to be investigated and their viability assessed in comparison with their diploid counterparts.
6. Evaluate biological and economical performance of triploids in different types of farming environments such as ponds (carp, European catfish), sea cages (seabass, seabream, cod), concrete raceways (sea bass, sea bream, turbot, halibut, freshwater salmonids, sturgeon) and recirculated systems (marine fishes). Evaluate also the organoleptic properties of triploids throughout the year.
7. Performance of induced sterile triploids in the natural environment should be investigated, taking into account possible species differences and sex, and addressing relevant traits such as survival, competition for resources and reproductive performance and behaviour.

8. Evaluate the ecological impact of the escape of small numbers of triploids from farms into the wild and the potential effects of restocking with large numbers of triploids.
9. If triploidy is going to be applied for the genetic containment of GMOs, then a reliable method is required for the low-cost, high-throughput, high-efficiency individual verification of ploidy and sterility.

Recommendations

1. Reinforce at all levels that induced polyploids are not GMOs.
2. Promote research to achieve sterility in triploids for the aquaculture industry.
3. For GMO containment purposes, 100% sterile triploid are required and must be verified on an individual animal basis.
4. For non-GMO production purposes, 100% sterility is less critical but protocols should be optimized.
5. The commercial application of triploidy should balance the advantages *vs.* the inconveniences on a species by species basis, since triploidy affects several characteristics of the animal (survival, growth, reproduction, etc.).
6. Tetraploidy is the common method for the induction of triploidy in shellfish and should be explored in other species. However, tetraploid stocks need to be contained.
7. Define and set up public information strategies (web sites, applied workshops, labelling, marketing approaches) between stakeholders in the aquaculture industry to disseminate the benefits and possible risks of triploidy.
8. Educate consumers by providing examples of how polyploidization has been used in agriculture for a long time, and how it is critical to current agriculture.

Suggested readings

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Applicability of gene transfer into the germinal line in fish culture

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Introduction

Transgenic organisms are one subset of genetically-modified organisms (GMOs) as defined by the European Union. Strains of transgenic fish have been developed in Asia, the Americas and Europe nearly 20 years ago, but many of the strains were not retained and commercial development of strains for aquaculture in Europe is currently minimal.

The failure of transgenic fish to be implemented into aquaculture has arisen for two main reasons: 1) significant social concern regarding food safety and environmental impacts which may arise from their use; and 2) despite encouraging data from laboratory studies (Fig.1, comparing normal salmon to those transformed with a growth hormone gene construct), a clear demonstration has not been made regarding the economic benefit of transgenic fish relative to existing strains which have been genetically improved by traditional methods, such as selective breeding. Nevertheless, internationally, research has continued in Asia and the Americas examining the commercial aspects and risk assessment of transgenic fish, and one company, Aquabounty in the USA, is applying to the US FDA for approval for marketing of the transgenic fish. This regulatory decision has been slow to be achieved, one speculates because of the complexity of the risk assessment task which accompanies such a decision. If approved, the application of these strains in large markets which do not require labelling has the potential to significantly shift the economics of salmon farming globally and could stimulate other jurisdiction to consider their implementation and to mediate pressure on international trade organizations which can regulate the sale of transgenic fish. Thus, scientific information needed to assist regulatory decisions is urgently required.

Fig. 1. *Normal (left) and GH-transgenic salmon (right) of the same age.*

Due to the current state of uncertainty associated with the use of transgenic organisms, coupled with their potential to assist in the efficient production of food at the global level, new knowledge is urgently required to clarify some of the major outstanding issues. Thus, Workpackage 1.3 of Workshop 1 of the EU Genimpact project has posed several questions to stimulate discussion of current issues associated with the use and safety of transgenic organisms. In the present context, transgenic organisms were considered as those containing novel gene constructs (i.e. homologous or heterologous DNA arranged in non-native forms) that had been stably integrated into the germline of fish. Several main areas were considered: 1) definition of genetically-modified organisms (GMOs); 2) issues associated with food safety; 3) environmental risks and reliability of experimental assessments of transgenic fish for hazard assessment; and 4) approaches for enhancing the biocontainment of transgenic fish.

From the discussion, research priorities have been suggested (see end) which could guide the acquisition of new knowledge and technology for the development and containment of transgenic technology.

Summary of responses to questions

1) Applicability of the substantial equivalence principle for the marketing of GH-transgenic fish

a) Is it appropriate that the current definition of the term "genetically modified organism (GMO)" be process-based rather than product-based?

In the EU, a process-based regulatory framework exists for evaluation of GMOs, which differs fundamentally from other global jurisdictions [*i.e.* Canada (*e.g.* Canadian Environmental Protection Act, CEPA 1999), USA and Australia] where regulations are based on characteristics of the "product". The rationale for using a product-based regulatory framework stems from the logic that it is the characteristics of the organism or its by-products which can result in health effects or environmental consequences. The nature of the process that generated two *otherwise identical* products does not influence the consequences of that product and therefore should not form the basis of regulatory scrutiny. Should identical strains produced through natural and anthropogenic means be regulated differently? Arguments for using a process-based regulation are derived from the consideration that certain processes, including transgenesis, may yield specific types of products which carry genetic changes (and thus create uncertainty and risk) that other approaches do not (such changes may or may not be identifiable with current technology; *e.g.* hypothesized trans effects on genes distant from the site of transgene insertion). It was noted that many natural processes (*e.g.* selective breeding, mobilization of transposons, etc.) also can yield many genetic changes which are not easily detected with current technology, but which may influence phenotype in both complex and simple ways. Significant discussion occurred within the group regarding an appropriate definition of what constitutes a genetically-modified organism. The definition ranges widely among researchers, from changes induced by altering the frequency of natural alleles in selected populations, to polyploidy and hybridization, to the transfer of non-integrated DNA into somatic cells of fish, or, in the strictest definition, to only those organisms that have been permanently transformed in their germ lines with DNA from a heterologous source.

GMO has been defined by the EU as «Genetically modified organism (GMO) means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination» (see below). In some jurisdictions, such as Canada, transgenic organisms are covered under regulation of "novel" organisms, *i.e.* those which have characteristics that have not previously been introduced into nature. It is recommended that further clarification of the use of the terms GMO (Genetically modified (or manipulated) organism), GEO (genetically engineered (or enhanced or enriched) organism), and novel organism, be undertaken in the EU.

A discussion also occurred regarding the use of substantial equivalence as an approach for regulating GMOs. Arguments for the approach suggest that, for example, expression of a host gene sequence (such as isospecific or homospecific growth hormone) may only quantitatively alter the characteristics of the organism, and does not affect the organism qualitatively. Arguments against this approach cite the potential of undetected changes to be generated by transgenesis that could yield novel protein products that may induce health or ecosystem effects, and that full analysis of the transcriptome and proteome are required to assess whether changes have occurred (it is noted that full transcriptome and proteome analyses are not yet possible for fish).

An alternate approach to using substantial equivalence is to adopt food safety evaluations used for all novel foods including those for organism which have altered level of a host protein, or a novel protein not normally present in the organism. Such tests would include allergenicity tests and multi-generational rat feeding trials followed by pathology tests and health status evaluations to search for *significant* effects on mammalian health. Such evaluations are likely to detect the vast majority of *acute* effects that have been hypothesized to be able to arise from transgenesis. Only long-term use of any novel product in a recipient population is likely to discover more subtle consequences, if they exists (analogous to the discovery of side-effects in most approved drugs following approval and wide-spread use in the population).

b) Is it appropriate that the current concern about GMOs be focussed on genetic changes rather than phenotypic changes?

Regulations should be based on the molecular, physiological and behavioural phenotypes associated with the organism, and most importantly how these characteristics differ from those found in wild type or other reference strains. Such differences then need evaluation with regard to food safety requirements and ecological consequences using the best methods available. Genetic changes are important with respect to how they may influence expression (*e.g.*, through position effects arising from characteristics of the specific site of integration) and stability (*e.g.*, is the site prone to deletion due to flanking repetitive elements, or alteration in copy number arising from changes in construct number which are organized in tandem arrays ?).

c) Since transfer of multiple foreign genes can spontaneously occur by introgressive hybridization, why introgressed fish are not to be considered as natural GMOs?

In many cases, hybrids are not novel to the environment, having arisen through natural interbreeding between species. Such hybrids contain genes and gene arrangements that have previously undergone natural selection in the wild. Although their genes now may be expressed in a novel nuclear environment, natural hybrids have had the historical chance to transfer genetic information. The consequences

of such exchange have further been subjected to natural selection. In cases where larger numbers of such hybrids are anticipated to enter an ecosystem through release or escape, then consideration of the effects of backcrossing with the parental species or further hybridization events should indeed be considered (*i.e.*, to evaluate the potential to significantly alter the population genetic structure of the resident population(s)). If the hybrids involve species that have not naturally hybridized, or they have the potential to enter novel environments, then careful examination of the characteristics of the organisms should be undertaken as would be performed for any novel organism (*e.g.*, introduced nonindigenous species).

d) Why were GMOs not regulated according to the same practice as at least fifteen fast-growing, fertile interspecific hybrids, which are currently admitted for fish culture despite the risk of back-crossing?

See c) above. For some hybrids, there may already be significant information available regarding their fitness in nature, which can assist with regulatory decisions about their potential consequences following introduction.

e) Is an aquatic organism bearing a construct encoding an isospecific protein eutopically expressed still to be defined as a GMO or rather as a "genetically enriched organism (GEO)"?

The type of protein expressed from a gene construct is only one part of the genetic information contained within it. Other critical genetic characteristics of the construct include regulatory changes which influence developmental timing of expression, tissue specificity, and quantity of the gene product. Each of these features has the potential to alter an organism significantly from its wild-type state. Altered tissue-specific expression can qualitatively influence the characteristics of an organism (*e.g.* from a food safety perspective, expressing a protein in muscle that is normally only found in liver, may alter the food characteristics of the flesh for human consumption). Altered levels of a protein can lead to downstream effects of other protein regulated by the transgene product (*e.g.* GH is known to stimulate IGF-I, a highly conserved vertebrate growth promoter) or may affect physiological process (*e.g.* thyroid hormone metabolism) or behaviour (*e.g.* GH is known to be a potent stimulator of feeding behaviour, which has a strong effect on the fitness of organisms in nature).

f) Are sterile transgenic fish, that cannot vertically transmit their transgene, still to be classified as GMOs? Is transformation of a functional germ line not required?

Yes, such organisms are still very much genetically modified in that they contain novel genetic information in their somatic and (in this case non-functional) germline tissues. Their entry into nature may still result in effects on ecosystem function over their lifetime. However, estimating such direct effects is vastly easier than the case where sexual reproduction can occur and result in the generation of new transgenic individuals in nature.

g) Do fast-growing fish bearing an isospecific GH-encoding construct satisfy the substantial equivalence principle for human consumption, because their protein profile has only been quantitatively modified? Should their sale still be subjected to stricter regulations than normal fish?

See a) and e). Quantitative changes can lead to qualitative changes, and to alterations in physiology and behaviour outside of the norm observed in the species. The effects

of overexpression depends very much on whether the direct and downstream effects have altered the product quality from that found within the normal range. If GH levels (amount produced, not the amount present in plasma) have not been raised beyond a level that is found within the normal range of already-consumed strains of the same species, then substantial equivalence may be considered. Arguments against substantial equivalence suggest that unidentified alterations may also have accompanied the known transgene insertion, and that undetected health/ecosystem impacts may well also exist. Empirical evidence demonstrating such hazards need to be carefully documented and scrutinized to ensure that the precautionary principle is not unnecessarily applied by infinite risks hypotheses.

2) Environmental safety of non-sterile transgenic fish and possible scenarios of transgene contamination of wild fish populations

a) How urgent is the approval of GH-transgenic fish for fish culture to meet future aquafood demands?

Discussion included which species are important to assist in global food production, where the food should be produced, and whether the technology should be developed in the EU to be provided elsewhere for application. The workshop attendees felt that transgenic salmon were not useful for this purpose, and that genetic engineering of this species primarily has the target of enhancing commercial efficiency of production and commercial benefit, and would be unlikely to significantly assist in providing animal protein for human consumption in developing nations. Other species such as tilapia and carp were recognized to have significant potential to assist in world animal protein production, and that enhancement of strains of these species by transgenesis could be of use. Characteristics felt important included enhancing food utilization (feed conversion efficiency), enhancing disease resistance, modifying the product characteristics (i.e. to make a healthier product with superior essential lipids and amino acid profiles), and improving abilities to utilize plant-based feed ingredients. Utility of transgenic technology also urgently requires better assessments of the benefits of GH transgenic fish under production conditions. Despite the apparent gains observed in laboratory trials of transgenic fish (particularly with GH), it was noted that few data currently exist regarding their utility in aquaculture environments (*vs*. the lab), and that comparisons to the best available domesticated strains is critical to evaluate their efficacy for enhancing food production. The use of fish species which can utilize vegetarian diets was also noted to be beneficial to those requiring fish/animal meals and oils which are now becoming very limiting.

b) Should the transfer of a construct allowing allopatric invasiveness be prohibited at all times similarly to the introduction of alien species?

Many constructs can be anticipated to alter the biogeographical range of a species, mediated through physiological enhancement of characteristics (*e.g.* ability to use nutritionally inferior prey items found in distant habitats) or addition of novel traits (*e.g.* antifreeze proteins which can confer enhanced cold tolerance and thus ability to invade new territories). In few cases, whenever the exact ability of a transgenic organism to possess enhanced host range is known, decisions regarding its risk to the environment should be determined from empirical studies examining its phenotype (*e.g.* thermal tolerance studies, nutrition trials, etc.).

c) Since private companies around the world are developing at least 20 species of transgenic fish and shellfish, should the choice of species be profit-oriented for private enterprises and relief-oriented for public agencies?

Both public and private research organizations can contribute both to private enterprises and global assistance objectives. Some felt that public funding should not be used to support transgenic research of any form, whereas other felt that such support could be used to develop technology which could be provided to both sectors. Others felt that this was not a question that was appropriate for science to decide. Political institutions and social policy should rather influence or make these decisions, that may vary among jurisdictions and over time. It was also noted that "private" transgenic organisms could be licensed by public entities to provide for relief purposes.

d) How realistic is the "Trojan gene hypothesis" by Muir and Howard (1999) assuming that GH-transgenic escapees with a mating advantage due to their bigger size but affected by inferior environmental adaptation might cause the extinction of wild populations?

The Trojan Gene (TG) hypothesis was acknowledged to be theoretically possible under specific stable conditions. A parallel was drawn to an extinction vortex proposed to occur from hybridization of wild fish with less fit domesticated genotypes. A difference noted with the TG hypothesis is that this scenario predicts that entire species extinction may arise by the introduction of, theoretically, a single transgenic individual (with the appropriate pair of opposing fitness traits) into a population, whereas an extinction vortex caused by interbreeding between wild and domesticated strains requires either sustained introduction of domesticated stock or the introduction of a very large population of domestic strain, which effectively eliminates the wild population genetics of the species. In the absence of a sustained input of a domestic stock, data are unavailable to determine whether such impacted populations may re-establish their natural allele frequencies which had been achieved through natural selection for high fitness in the wild. Factors which would limit the likelihood of the TG hypothesis from becoming a reality in nature include: 1) the extinction effects only occur under a restricted range of fitness conditions; 2) natural selection of background genetic variation will shift fitness values over time, resulting in diminution of the negative fitness trait away from that required for a TG effect; and 3) to date, GH fish do not always show enhanced body size and mating advantage relative to nontransgenics.

e) How realistic is the assumption that the growth enhancement promoted by GH-construct transfer would also be associated with greater predatory capacity in transgenic escapees in the wild?

GH transgenic fish that have been tested display significantly enhanced feeding behaviour and prey capture rates. In trials where transgenic and nontransgenic fish are in competition, transgenic fish consistently acquire food resources preferentially over controls. In nature, however, enhanced feeding ability does not necessarily translate into enhanced fitness, since fish in many cases must trade off the benefits of foraging activity with the risk of being subjected to predation themselves. Thus, growth rates of fish in nature are not often maximal, but rather have been selected to provide maximum fitness. GH transgenic individuals have had their feeding behaviour forcibly altered away from a naturally-selected, presumably, optimum. In semi-natural environments, where transgenic fish are foraging for natural prey items, it has been found that they succumb to predation mortality at a greater rate than controls and thus display lower fitness. It has also been found that populations containing GH transgenic are less able to withstand periods of food shortage, resulting in population crashes. Developmental rate has also been found to affect

the fitness of transgenic fish, such that early emergence from natal redds has been shown to result in preferential predation mortality.

f) A non-indigenous species that has become naturalized after its introduction (e.g. Nile tilapia or its hybrids) is a better candidate for gene transfer than an indigenous species?

A naturalized species may have a better chance of survival in nature and thus causing effects on other organisms. Using highly domesticated strains with poor natural fitness (but high cultured fitness) that have no counterparts to breed with in the vicinity may be useful for containment. The use of monosex strains or sterile hybrids also could provide high level containment for some species, where no conspecifics or other related species are present in the ecosystem to breed with.

g) Are fish feeding at the bottom of the trophic chain (phytoplanktophagous, vegetarian) better candidates for increasing world aquafood production by means of gene transfer than carnivorous species? Is the issue of sustainability relevant to transgenesis application in fish?

Farming of fish that have high production efficiency should be the goal for world fish production, using feed ingredients derived from as close to sunlight as possible better (*i.e.* plants, algae). Such species may have a greater potential to alter food webs, however, and cause environmental damage, depending on the exact species they are capable of harvesting from the environment. This must be assessed on a case-by-case basis.

h) Why is transfer of foreign non-coding sequences (eg. satellite DNA) into wild conspecific populations by crossing with domesticated escapees not considered as a gene transfer? The phylogenetic record of the wild population is certainly perturbed.

If this transfer can occur through natural hybridization, then the resulting organism is not novel to the environment. If it is something not seen before in nature, then some jurisdictions would consider it a GMO (or novel) which would require evaluation. Thus, the current definition of GMO by the EU may require some modification.

i) Should genetic mutations induced by genotoxic substances discharged with industrial effluents in inland water bodies or marine coasts be regarded as true "genetic modifications" and the affected organisms classified as covert GMOs? What about the ecosystemic damage due to the transgenerational increase of the genetic load in wild populations?

Yes, all genetic changes that may produce a novel phenotype should be examined. But this would be a very large task to undertake. Disruptions of the natural population genetic structure of fish is an important impact that needs to be evaluated during a risk assessment.

3) Feasibility and reliability of recommended field tests to assess the environmental safety of transgenic fish

a) What qualifications should the experts in charge of developing legislation for transgenic aquatic organisms have?

Research experience in biological sciences (genetics, molecular biology and ecology), and experience in Risk Assessment methodology and Decision Analysis, as well as legal experience are required to provide a reliable risk assessment.

b) How should field tests be carried out to avoid dispersal of tested transgenics?

Fish released to natural ecosystems are essentially unrecoverable. Thus, if the result is subsequently determined to be detrimental to the environment (*e.g.* Nile Perch in Lake Victoria), it is unlikely that recovery will be possible. Thus, the use of laboratory apparatus, semi-natural mesocosms, and modelling are alternatives that can provide useful data of phenotypic effects. An alternate approach is to utilize surrogate systems to mimic transgenic animals. For example, slow-release formulations of fish GH can be implanted into fish which can be released to nature without risk of sustained genetic consequences. Similarly, although as yet untried, individually-verified triploid sterile animals introduced into contained aquatic natural environments may be developed with minimal potential for long-term environmental consequences.

c) Is it legitimate to extrapolate results about competitiveness of transgenics in a secluded biotope to the different situations of open environments?

Results to date examining the traits of transgenic and control animals in the laboratory have revealed that there are very strong environmental effects on phenotype (phenotypic plasticity). More importantly, this plasticity is subject to genotype by environment (GxE) interactions, which results in nonlinear reaction norms for trait character across different environments. Such results strongly suggest that data acquired in the laboratory, even under the most complex apparatus that can be developed, are likely to fall short of accurately representing the full spectrum of environments that a transgenic fish is likely to encounter in nature. Certainly all effort should be made to cover a broad range of conditions, from which sensitivity analysis may be used in models to assist in predicting critical controlling parameters. However, definitive identification of the true fitness of a transgenic animals and its consequences cannot easily be determined from lab studies. Due to these uncertainties, biocontainment strategies are urgently required.

d) How transparent can a field test be carried out within the internal facilities of a private company? What if activists opposing GMOs infiltrate a public facility for transgenic tests?

Ideally, all pertinent risk assessment information should be published in the peer-reviewed literature. There is also a benefit of public research programs and model strains of transgenic fish which allow (open) access to perform studies (in contained facilities) and publication of information regarding the benefits and risk of transgenic fish technology. Hence, everybody can (openly) evaluate and challenge the GMOs using scientific methods.

e) Is the requirement of field tests actually a demand for a never-ending sequel of expensive trials of dubious significance, prone to conflicting results and exposed to the enforcement of a strong precautionary principle with oppressive bureaucracy? Or are they absolutely necessary?

To obtain *true fitness and consequence* data, field tests are the only possible approach. If contemplated, field trials should be performed only after lab and semi natural mesocosm studies assessing phenotype and consequence have been performed, and should be conducted only with biologically-contained animals (which limits the level of impact that can be studied). Currently, the uncertainty associated with transgenic fish fitness and consequence in nature are too great to support field trials, given the inability to recover such fish should an introduction with negative consequences ensue.

f) Should field tests be conveniently replaced by straightforward anatomical, histological and functional fertility tests, implying that all transgenics must be proved as reproductively incompetent?

Sterility can be confirmed in individual fish using triploidy analysis (*i.e.* flow cytometry of blood cells). This method is currently used successfully to introduce sterile grass carp for weed control in the USA. The application of triploidy testing for aquaculture is not likely to be economically viable on a large scale due to the requirement for handling and analysis of each fish. Alternative methods of biological containment are required. Private companies may accept this requirement, which would confer them full market control on their product without the risks of being sued at later times for unforeseen transgenic contaminations. Sterilization technology provides convergence of ecological and economic benefits associated with the application of transgenic fish.

4) Technical approaches to achieve full biological containment of farmed transgenic fish

a) Is the transfer of a construct encoding anti-sense mRNA for GnRH an adequate measure to ensure complete sterility during growout of transgenic fish?

To date, this approach has resulted only in partial sterility. Other approaches including RNAi and targeting all GnRH loci in the genome, may prove to be more effective. Full restoration of maturation and fertility by treatment with exogenous GnRH peptide must also be demonstrated in the sterile strains for the technology to be effective.

b) Can direct and indirect techniques for triploidy induction secure complete sterility of transgenic fish and shellfish?

Temperature and pressure shock-induced triploidy in finfish does not yield 100% triploid populations of animals. Experiments on large numbers of transgenic coho salmon have shown that while high levels of triploidy can be achieved (99.8%) with pressure shocking, varying the treatment conditions (*i.e.* temperature, shock pressure, timing, and duration, age of eggs, time post fertilization) does not eliminate diploid exceptions for treated groups. Given the large number of fish which can escape from aquaculture facilities, even a frequency of 0.2% could allow the introduction of a significant number of fertile animals into natural ecosystems. The use of tetraploidy may enhance triploid production; however, it should be noted that tetraploid strains generally have impaired viability, and evidence has been presented indicating that unreduced gametes can arise from tetraploid strains (which would result in pentaploid rather than triploid offspring). Nevertheless, this is a promising area of research which should be further explored.

c) Are sterile fish hybrids obtained by intergeneric hybridization of transgenic pure species safe candidates for mass culture? Should distant hybridization be associated with triploidy induction to produce sterile transgenic allotriploids with adequate biocontainment?

Such hybrids if reliably sterile as diploids, or inviable as diploids but sterile as triploids, could be of extreme value, particularly if coupled with triploidy. This approach could provide backup containment if the triploidy induction process failed. This approach would need to be evaluated on a large scale to examine rare meiotic events which may yield gynogenetic and/or aneuploid offspring.

d) Should transgenic fish be raised only in closed recirculating systems?

If biological containment cannot be achieved, then effective containment can be achieved using land-based facilities. The economic benefit associated with the enhanced production trait of the transgenic strain would need to offset the significant investment required for land-based aquaculture. Protection against predictable catastrophes (hurricanes, floods, earthquake) also needs to be considered in this case (*e.g.* carp escapes from ponds in Europe).

e) Are techniques available for crippling transgenic fish in order to make them unable to survive in the wild?

There are a variety of genetic and molecular biology approaches which can be developed to enhance biological containment. Developing knockout technology (*e.g.* site directed mutagenesis, TILLING, and RNAi methods) could allow development of strains which are dependant on supplemental compounds for survival and or reproduction. These techniques are in their infancy, and should be supported since they have the ability to provide solutions for containment not only of transgenic fish, but also of nonindigenous species and selectively-bred native strains. If transgenic approaches are utilized, these too would require separate risk assessments.

f) Has any country provisionally approved transgenic fish for human consumption?

The group is unaware of any transgenic fish being used in aquaculture at this time, despite several strains apparently ready for deployment should approval be granted by regulatory agencies.

Research Priorities

1. Research examining the novelty (physiological, morphological, and behavioural) of different forms of genetic modification using molecular tools such as DNA microarray, proteomics and QTL mapping are required to reveal the underlying basis of phenotypic differentiation among strains. For example, are growth-enhanced and domesticated fish similar enough to be regulated in the same way?
2. Knowledge on how genetic and epigenetic changes influence the expression of transgenes and thus the phenotype of the modified organism is needed to evaluate the long-term stability of GMOs, and also their response to environmental variables (phenotypic plasticity and GxE effects). Understanding phenotypic plasticity of traits, and most importantly genotype by environment interactions, is at the key of evaluating the reliability of risk assessments. It will hardly be possible to provide reliable phenotypic data on different transgenic and wild type strains, which vary under different environmental conditions, for robust predictions in nature.
3. Hybridization should be examined for its ability to create novel phenotypes not previously present within an ecosystem. Both production traits and those suspected of being associated with ecological risk should be examined. In particular, comparison of hybrids which have experienced historical introgression events and those that are novel could be revealing.
4. Detailed genetic, molecular, physiological and behavioural studies should be undertaken with defined transgenic lines to examine the extent of indirect change which may have arisen as a consequence of transgene expression. Comparison

of several systems (*i.e.* different species, transgenes) which contain homologous or heterologous proteins expressed at different levels, times, and tissues should be examined.

5. The phenotypic characteristics of sterile transgenic fish should be examined for both their production characteristics and for their ability to interact and potentially impact the ecosystem directly throughout their lifetime.
6. Economic and sociological studies examining current data are needed to assess the true potential of genetic engineering (*vs*. alternative approaches) regarding the applicability of transgenesis to enhance food production efficiency in simulated field situations. Research developing genetic lines which utilize available and local plant-based protein meals and oils would be of great benefit.
7. The Trojan Gene Hypothesis, although theoretically possible, needs to be tested with rigorously-defined transgenic strains with significant growth enhancement. To date, examination of this hypothesis has been accomplished through modelling and a single poorly-defined ornamental model fish species. It is likely that this scenario is very case-specific.
8. Data indicate enhanced feeding behaviour of GH transgenic fish, but that this is accompanied by enhanced predation risk. The small amount of data available does not allow extrapolation of the lab-based information to the field. More complex apparatus and experiments are needed examining critical ecological variables (density, food availability and type, interactions with pathogens, etc.). Examining scenarios through multiple trophic levels would be of relevance to determine whether cascade effects are likely.
9. Research in nature is required to accurately determine the risks of releases of transgenic fish into nature. Since such releases are not allowed, estimates of fitness effects and ecological consequences must be obtained from confined laboratory studies. Research should be encouraged which examines the genetic and phenotypic characteristics of transgenic fish in laboratory scenarios, in mesocosms simulating to the greatest degree possible natural environments, and from surrogate systems (*e.g*. implantation of bioactive compounds which mimic transgenic phenotypes).
10. Research is required to understand the pleiotropic nature of transgenic phenotypes and their possible effects on the fitness of the transformed species. While currently it can be very difficult to extrapolate laboratory phenotypes to ecological consequences, laboratory studies are still needed to identify the basic phenotypes of the transformed organisms from which more complex experiments may be designed.
11. An additional long-term problem associated with risk assessments is the changing background genetics interacting with the transgene which will be selected over time. Observations have suggested that genetic background can affect the phenotype of transgenic fish, which indicates that, in nature, a transgene experiencing natural genetic variation within and among populations would likely shift phenotype in response to natural selection. This makes risk assessments a moving target. Thus, understanding the genomic control of transgene expression is critical.
12. New methods need to be developed for enhancing the efficacy of biological containment. Approaches include: 1) improvements of polyploidization procedures (*e.g*. efficacy of triploidy production through optimizing conditions

and/or production of tetraploid strains); 2) use of hybridization (directly if the hybrid is sterile, or in combination with triploidy if the diploid hybrid is lethal); 3) development of transgenic approaches to inducing sterilization or inviability; and 4) development of non-transgenic methods for inducing genetic changes allowing biological containment.

13. Research examining the economic feasibility of land-based aquaculture of genetically-enhanced strains could assist in decisions regarding the applicability of the technology for developed and developing countries.

14. Research on enhancing physical containment through improved engineering of culture facilities.

Recommendations

1. The current definition of the term "genetically modified organism" by EU should be reconsidered because it disregards organisms which are genetically modified by powerful genetic techniques, such as induced mutagenesis, is biased by the implicit assumption that genetic modification by natural mating and/or recombination is of less concern as opposed to transgenesis (invalid in the case of alien species), and does not distinguish between autotransgenesis with intraspecific gene transfer *vs*. allotransgenesis with interspecific gene transfer (see also annex).

2. Careful consideration should be paid as to whether there is a real safety problem posed to consumers by fish and crustaceans fed on diets containing transgenic plant ingredients. In particular, regulations about safe levels should not be arbitrarily fixed, but based on experimental evidence.

3. Evaluation of the possible promotional abuse of the term GMO-free (neither GMO *per se* nor GMO-fed) to improve the image of processed fish products that have no other truly enhanced quality or merit.

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DNA vaccination in fish culture

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Introduction

The first vaccines against infectious bacterial diseases in farmed fish were developed in the 1970s and applied on a commercial scale in the early 1980s. Development of DNA vaccines only started experimentally in the early 1990s and there is only limited experience about their effectiveness and adverse effects in fish. DNA vaccination is based on the administration of a plasmid encoding the vaccine antigen, rather than the antigen itself. The latter is the case in polyantigen-vaccines, such as attenuated bacteria and viruses or bacterial capsule polysaccharides and toxins, as well as in monoantigen-vaccines based mainly on recombinants viral proteins. Expression of the plasmid in the somatic cells of the host triggers both its humoral and cellular immune responses.

The prophylactic potential of DNA vaccination in fish culture rests on several advantages, including identical and inexpensive production processes, the possibility of co-administration of multiple vaccines (multivalency), simplicity of storage due to the high chemical stability of plasmid DNA, rapid modification of vaccine DNA sequences to confront new pathogen mutants, no risk of disease transmission (as occurs with live attenuated vaccines), proper conformational folding of the antigen protein of the pathogen (not always achieved with recombinant protein vaccines produced in bacteria), no need for adjuvant use and boosting to elicit immune responses, and effectiveness in stimulating both humoral and cell-mediated immunity (Heppell and Davis, 2000). Despite unsolved technical and biological problems regarding a suitable administration method and unwanted integration of the plasmid DNA into the host genome at the germinal line, it is expected that these difficulties will be overcome, permitting the widespread use of DNA vaccines as a viable vaccination method in the future.

Summary of responses to questions

1) Comparison of DNA vaccines vs. antigen vaccines

a) Should the introduction of DNA vaccines in fish culture be regarded as an urgent issue? Do they provide prophylactic effects not covered by antigen vaccines?

Yes to both. DNA vaccines activate arms of the immune system that recombinant antigens cannot mobilize.

a) Is the adoption of DNA vaccines in humans and tetrapods a suitable argument for licensing their use also in fish?

No, because there may be important immune system differences, and because any DNA vaccine must be tested case by case in the relevant host and environment.

b) What are the main differences in DNA vaccine effectiveness in fish and tetrapods?

No general conclusion. This is open for investigations.

c) Are DNA-medicated fish to be considered as genetically modified organisms

(GMOs)? What about DNA-vaccinated patients?

No conclusive opinion. Depends on the definition of a GMO.

d) Are DNA vaccines to be objected on the same precautionary grounds as attenuated virus vaccines?

No, not on the same grounds. There are different risk issues to be considered.

f) What are the drawbacks if DNA vaccines are not allowed for commercial use in fish?

For some diseases, we may not have any other good alternatives.

g) What problems would cause divergent regulations for DNA vaccines by different countries on the international trade of cultured fish?

The question anticipates possible applications, but it is difficult to forecast future scenarios.

h) Should a DNA-vaccinated fish at market size satisfy a labelling requirement? What about labelling of antigen-vaccinated fish?

We lack the research results necessary to give a definite answer.

2) Development of suitable methods for mass administration of DNA vaccines in fish

a) Is the DNA vaccine technology adaptable for mass-scale applications in fish culture?

Yes, provided that multivalent DNA vaccines can be made.

b) Are vaccination machines for intraperitoneal delivery already operated commercially adaptable for i.m injection of DNA vaccines in fish?

Only further development and experiments can tell.

c) Is mass boosting of DNA vaccines feasible in fish, if required? Can boosting be eliminated in DNA vaccination by one-shot administration with genetic or hormonal immunostimulants or chemical adjuvants?

No, for practical reasons. As for biological stimulants and chemical adjuvants, there is a lot of research needed before safe and efficient use.

d) What are the alternative administration routes to i.m. injection? Pros and cons of biolistic methods (gene gun, ultrasound-pressured DNA-coated microspheres), micro-encapsulation (biodegradable, non-antigenic polylactide or polyglycolide (PLGA or PLG) microspheres), lipofaction (liposomes) and immersion or spraying.

No definite answer. Has to be tested case by case with combination of methods and DNA constructs.

e) What damage is caused to fish by DNA vaccination as compared to antigen vaccination? How extensive is the cytotoxic destruction of myocytes superficially exposing fragments of the encoded antigen?

Less local tissue destruction with DNA vaccines, generally speaking. However, this may vary from construct to construct.

f) What about employees' safety when administering DNA vaccines by injection?

Risks of being immunized by inadvertent puncture?

Prevention of contamination by adequate protection measures required. Consequences of unwanted immunological and autoimmune reactions (*e.g.* lupus) may, case by case, to some extent be clarified by animal experiments.

g) Should plasmids for mass vaccination be devoid of encoded antibiotic resistance?

That is definitely a wanted goal.

3) Persistence, fate and safety of DNA vaccines in fish

a) How effectively are plasmids taken up by skeletal myocytes after injection?

In mammals, there are unexplained differences between different DNA constructs. This is an area where much more research is needed. If generic expression plasmids are efficiently taken up, this would be of great advantage.

b) Can plasmids associated with transfection agents (liposomes or microcarriers) be taken up by fish skin or gills? What is the efficiency as compared to i.m. injection?

Open area for research. Conflicting results in the literature.

c) Are antibodies formed in fish against double-stranded DNA? Are they pathogenic?

If plasmids are complexed with immunogenic proteins, antibodies against dsDNA may be produced. The consequences are unpredictable.

d) What is the persistence of episomal plasmids in vaccinated fish?

This is obviously an area of omitted research. Experimental research in salmon has indicated persistence of plasmids, transcripts and reporter proteins at the i.m. injection site for 1.5 years. The results may be determined by the construct and has to be tested case by case. This question deserves more research.

e) What is the prevalence of local and spread-out integration of plasmids into the host genome?

There are very conflicting results in mammalian organisms as well as in fish. It is probably varying with DNA constructs and fish species.

f) Should intraperitoneal injection of plasmids be avoided because of risk of integration into the germinal line?

This can only be settled by further research.

g) What are the expected consequences of plasmid integration into the germinal line and vertical transmission to the offspring?

Various worst case scenarios may be given. Can only be answered by further research.

h) Is the concern about potential spread of vaccine DNA into the environment by predators that eat vaccinated escapees justified?

Answers can only be based on assumptions. Can only be answered by further research.

i) Can residual vaccine plasmids in eaten fish be transferred into the intestinal flora of the consumer or be taken up by enterocytes or immune cells in the gut?

What about the bacterial plasmid content of the normal food?

There are only results from a couple of animal and human volunteer studies to extrapolate from. The probability and the consequences are unknown. There may be a difference between naturally occurring plasmids and DNA constructs. The end result is determined by context.

4) Feasibility of simultaneous DNA vaccination against multiple strains of a pathogen or against several pathogens

a) Is mixing DNA vaccines with antigen vaccines a promising strategy in fish?

No science-based answer can be given. In mammalian organisms initial immunization with DNA vaccine and boosting by another formulation may be favourable. Open to research.

b) For multivalent DNA vaccination, what is the difference between colinear expression of different antigens in the same plasmid vs. coinjection of multiple plasmids?

Multi-cDNA plasmids may be difficult to construct, while multi-epitope coding plasmids may be a practical possibility. Should be open to research.

c) What are the risks of DNA vaccine administration to incompletely immunocompetent young fish? May they become totally susceptible to the pathogen because the antigen is recognized as a self component later on?

This is an open question. In practical terms, the age of immunocompetence must be known. From mammalian systems, the scenario presented has been observed. Furthermore, transfer of maternal antibodies in fish is an unknown area.

d) Can the prolonged expression of a plasmid-encoded antigen cause antigen tolerance in fish, with the risk that they may become asymptomatic carriers for the pathogen?

If the immune response is not adequate, with "healthy" viral carriers is a distinct possibility. The combination of DNA construct and the life history of the corresponding pathogen may be decisive. More research needed.

e) Can fish vertically infected through the gametes, even at a very low level, become totally unresponsive to DNA vaccination at the fingerling stage (eg. *enzootic -nodavirus)?*

Interesting question. Deserves more research.

f) May consumers' aversion against GMOs influence the acceptance of DNA-vaccinated fish? Can such a damage to the image of vaccinated fish be overcome?

The public perception of the biosafety-related research is a decisive factor. If the public is convinced that vaccination contributes to healthier fish, and that healthier fish is healthier to consume, "aversion" may be turned into acceptance.

Research priorities

1. Promote research on the development of immunocompetence in different cultured fish species.
2. Since DNA vaccination poses fewer risks than vaccination with attenuated viruses, identification of conserved regions in the genetic encoding of superficial

proteins is needed to overcome haplotypic divergence of current strains.

3. The effectiveness of DNA vaccination in fish species exposed to the vertical transmission of viral pathogens (*e.g.* b-nodavirus) should be investigated and the requirement of stamping out of contaminated broodstock established.
4. Development of suitable delivery strategies for mass vaccination of small fish is critically needed and should be a parallel research line with respect to DNA vaccine design and production.
5. Research is required on the fate of the DNA vaccine in the host fish, in terms of level of expression, persistence, mobilization, risk of genomic integration and transmission in the germinal line.
6. Research is required about possible adverse effects of plasmids in fish, the environmental risks associated with their administration on a commercial scale and safety concerns about consumers.

Recommendations

1. Present regulations lack clarity in distinguishing DNA-vaccinated animals from genetically modified organisms (GMOs), which could raise issues in terms of licensing and public acceptance of the technology.
2. Evaluate whether the lack of labelling requirement for antigen vaccination in fish can be extended to DNA vaccination as well.
3. Promote the development of DNA vaccination techniques that ensure a long-lasting protective immune response, while restricting the persistence of introduced DNA to the time required for that response.
4. Given the risks and costs of vaccination with attenuated viruses and the lack of cellular immune responses with recombinant protein vaccines, promote collaboration between stakeholders in the aquaculture industry and research centres to accelerate the development of DNA vaccines for mass scale applications to cope with difficult to eradicate viral enzootics (*e.g.* viral diseases affecting fresh water fishes, like salmonids, catfishes and cyprinids) or expanding viral epizootics (*e.g.* b-nodavirosis targeting a multitude of marine teleost species).

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The semantics of the term "genetically modified organism"

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Owing to the difficulties in properly delimiting the meaning of the term "genetically modified organism" (GMO), as applicable to transgenic fish, the following critical considerations on EU regulation 1829 concerning the definition of GMO, are proposed, in order to better understand the assumptions from which a definition has been enucleated that is now of public use.

Regulation 1829

This Regulation provides uniform procedures throughout the EU for the assessment and authorisation for the use in the EU of GMOs and of feed made from or using GMOs or their derivatives. It also provides for the labelling of such food. Regulation 1829 was made on 22 September 2003. It was published in the official journal on 18 October 2003, came into force on 7 November 2003 and became legally binding with direct effect in the EU from 18 April 2004.

For the purposes of Regulation 1829, GMOs are defined as such within the meaning that has been given to that by Article 2(2) of Directive 2001/18/EC. Excluded from this are organisms obtained through the techniques of genetic modification set out in Annex 1B of that Directive.
That definition of GMOs is set out as follows.

Article 2 – Definitions

For the purposes of this Directive:

(1) organism means any biological entity capable of replication or transferring genetic material;

(2) genetically modified organism (GMO) means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;

Considerations

In this definition, the term "genetically modified organism" (GMO) is process-based rather than product-based (see above).

The focus of the definition is on the alteration of the genetic material *per se*, that is the genotype, without reference to the changes induced in the phenotype.
The definition allows easy screening of organisms between GMOs and non-GMOs according to the techniques involved.

Phenotypic changes are evaluated only in the risk-assessment phase in the organisms classified as GMOs.

The approval of GMOs for mass production or commercialization is conditioned by a favourable benefit/risk ratio.

The EU definition does not include:

- interspecific hybridization
- transfer of multiple foreign gene by introgressive hybridization;
- inbreeding for pure line formation
- induced polyploidization
- selection of spontaneous mutations
- induced mutations by genotoxic substances or irradiation
- viral transformation

However, there is the possibility of significant risks even with these techniques (*e.g.* generation of killer bees by crossing African and European strains; tetraploidy is a natural fast mode of speciation; mutations can be variably deleterious or harmful; viral vectors may become permanently integrated and vertically transmitted).

On the other hand, the definition would include:

- gene-knockout organisms (lacking a functional gene)
- organisms with DNA encoding anti-sense mRNA (impairing translation of endogenous mRNA)
- organisms subjected to dominant-negative technology (reducing the activity of a protein)
- diploid gynogenetic and androgenetic clones (totally homozygous and with ineffective allele recombination).

It should be emphasized that the threat is not posed by techniques, or by altered genetic information *per se*, but rather by the expression of this information into modified phenotypic traits. The product and not the process is really the source of risk (see above).

The definition should be more comprehensive with the inclusion of all techniques capable of producing significantly modified phenotypes.

On the other hand, the specification of GMO should be considered as a provisional notation, inasmuch as the terms “modification” or “alteration” do not indicate whether the introduced change is risky or not.

The risk assessment phase is, therefore, in charge of defining this aspect. In the case of GMOs for food, the recognition of the safe application of the “substantial equivalence principle”, the lack of foreseeable permanent ecosystemic impacts due to complete reproductive sterility, and obviously the association of some consumer benefits should imply the removal of the labelling as GM and its replacement by a positive notation, such as “genetically approved (GA)”, or a bar-code describing the whole evaluation and approval process. In other words, emphasis should be given to the terminal act of the process (the result of the risk evaluation) rather than the initial act that started the process (the generation of the GMO).

If the GMO production involves the use of less possible environmental and food contaminants, such as pesticides, herbicides, fungicides etc., or the acquisition of substantially better nutritional qualities, then the label should indicate this positive character with the notation "genetically improved" (GI).

The EU definition of GMO does not really specify whether all the genomic copies within an organism must be altered by integration of a foreign sequence or only a few of them. In the first case, DNA-vaccinated organisms would be excluded from GMOs, even if there is a remote risk of somatic genomic integration. Otherwise, somatic gene transfer will be equated to germinal gene transfer and DNA vaccination subjected to the same requirements (e.g. reproductive sterility) as canonical transgenesis.

If DNA-vaccinated fish are not to be considered as GMOs, by the same token, also mosaic transgenics should be excluded. Paradoxically, by *naturally* crossing a mosaic transgenic with a wild-type conspecific, a transgenic line homozygous for the transgene may be obtained by natural mating. These transgenics would not be classifiable as GMOs.

This impasse would be overcome by stating that, in GMOs, the gene transfer must affect the germinal cell line and that this should be functional in order to allow the vertical transmission of the transgene (non-gametogenic germinal cells are equivalent to somatic cells). In this case, fertile transgenics would qualify as GMOs, but totally sterile transgenics would not.

II

Monitoring tools for evaluation of genetic impact of aquaculture activities on wild populations

These chapters report an updated overview of the knowledge available on the existing genetic tools for identification of aquaculture individuals in the wild, as well as to assess their potential impacts on wild populations based on their fitness.

Monitoring tools for identification of aquaculture escapees/released individuals and their offspring, and for assessing their fitness, were reviewed for the 12 species/groups of species considered in Genimpact. Specific research priorities for the future are recommended.

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Assessment of tools for identifying the genetic origin of fish and monitoring their occurrence in the wild

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Introduction

The potential genetic effects of aquaculture activities have aroused a great deal of concern, and the perceived risks are often associated with interbreeding with natural populations and the adverse effects of ecosystem interactions (1). The EU-funded project Genimpact (http://genimpact.imr.no) reviews specific aspects of potential risks and concerns on interbreeding and aquaculture-ecosystem interactions. In workshop 2, emphasis was given on the current knowledge and state of art of the tools available for the study of monitoring escapees of the species under study i.e. Atlantic salmon, Atlantic cod, European sea bass, gilthead sea bream, turbot, common carp, Atlantic halibut, scallops, mussels, oysters (Pacific oyster and European flat oyster) and European lobster. Additionally emphasis was given on the future research objectives for better and improved monitoring methods.

Current knowledge on non genetic tools

Identification of escapees is assured if all farmed fish are tagged, however tagging may not always be a realistic option due to high cost or biological restrictions such as the size of the cultured individuals. The most commonly used non-genetic methods for discrimination among farmed and wild fish include: i) *External identification tags:* this has been widely practised since the dawn of aquaculture. The major problem has been the difficulty of tagging small fish; however recently, new methods using fluorescent implants have been used successfully; ii) *Morphology/Morphometry:* since farmed fish are often characterized by body changes or defects that can be used for visual detection by professionals or laymen; accuracy and consistency are however, in many cases uncertain; iii) *Scale and otolith pattern recognition:* growth patterns in scales and otoliths can be used for identification of farmed fish; and iv) *Biochemical and physiological markers:* such as carotenoids and fatty acids due to different diets of wild and reared fish, or analysis of trace elements in otoliths and bone structures; however, so far, most work has been performed to identify Atlantic salmon escapees and relatively little on the other species.

Current knowledge on genetic tools

In aquaculture, molecular markers are increasingly employed for purposes of monitoring genetic variation in domestic stocks and for identifying domestic individuals in the wild (2, 3). Molecular markers are inherent to the individual, thus they can't be lost. If polymerase chain reaction (PCR) is used to resolve variation, tissue sampling does not require animals to be killed. Their main advantage with respect to physical markers is that they are inheritable, enabling identification of the offspring of aquaculture individuals released or escaped into wild populations. Thus genetic markers allow the estimation of the true impact of aquaculture escapes in wild populations through fitness studies. The main genetic markers that have been used are allozymes, mitochondrial DNA and microsatellite DNA. Future markers include coding DNA variation based on single nucleotide polymorphisms (SNPs) and / or using DNA microarrays.

The first genetic tool to be used involved the analysis of inherited variation in proteins (enzymatic proteins) within and among wild and farmed fish and shellfish populations. The different allelic forms, referred to as allozymes, result from DNA base variation in protein coding genes which causes amino acid changes and changes in either the protein's electrical charge or its molecular shape. Some of the major problems of this method include i) that it generally requires destructive sampling of individuals to obtain required tissue samples, ii) that allozymes lose their activity very fast and therefore the maximum storage time for allozymes is much shorter than DNA samples, and iii) the variation resolved has proven of limited use for monitoring farm escapes, or for studying the genetic effects of cultured fish on wild populations. Of the species considered, the method has been most widely used in relation to the Atlantic salmon (4), though extended studies have also been done on carp. In general allozyme markers are now seldom employed and have been largely superseded by DNA based analyses.

Mitochondrial markers were the first DNA markers to be extensively used. Mitochondrial genomes, in animal species, consist of single circular pieces of double-strain DNA As the mitochondrial genome mostly contains coding DNA, its main source of variation is SNPs. The mutation rate is higher in mitochondrial than in nuclear coding DNA regions resulting in higher levels of variation and population differentiation. Significantly different levels of nucleotide diversity are detected among different mitochondrial genes and DNA regions with different levels of variation can be chosen as markers of different biological units: highly conserved regions, for developing species-specific or race-specific markers; less conserved regions, for markers of stocks, and so on. Additionally, in higher animals,mitochondria are typically inherited via females, therefore DNA cannot detect introgression via males, but can be used for exploring sex-biased gene flow. The only exceptions are mussels and other bivalves in which a second mitochondrial genome is paternally inherited (the M genome).

Microsatellites have, in the last decade, developed into the most popular genetic markers (2). Microsatellites or simple sequence repeats (SSRs) are tandemly repeated motifs of 1-6 DNA bases, which are abundantly distributed within genomes and usually characterized by a high degree of polymorphism in the number of repeats. With the advent of PCR technology, microsatellite loci can be easily amplified using specific primers which bind to the region flanking the variable microsatellite. Recently, with the availability of high-throughput capillary sequencers or mass spectrometry, the characterisation of variant types, once a bottleneck, has become

easy and rapid. By analysing a panel of multiple microsatellite loci, a unique combined SSR genotype profile can be produced for each individual tested and studies show that such genotype profiles are highly discriminating, with randomly chosen individuals having a low probability of having matching genotypes. In the field of fisheries and aquaculture, microsatellites are useful for the characterization of breeding populations and stocks, for paternity and relatedness analysis of natural populations, hatchery broodstock selection, constructing dense linkage maps, and mapping economically important quantitative traits. With this type of information and the development of powerful analytical methods/statistical programmes in recent years, the focus has increasingly shifted from defining populations to discriminating individuals (5); it is now often possible to assign or exclude individuals originating from a claimed population. This methodology has applications to the identification of the genetic origin of specific individuals, of immigrants into populations, the occurrence of hybridization or admixture, the assessment of introgression of hatchery individuals into wild populations, and evaluation of the success of stock enhancement programmes.

More recently efforts have focused on direct analysis of coding DNA. This encompasses the small fraction (normally 5% or less) of the genome of any higher organism which is transcribed and used to produce messenger RNA, much of which is further translated to produce proteins. Variation in coding DNA is generally assessed by means of DNA sequencing and most commonly involves SNPs; these are individual point mutations in genomic DNA at which different sequence alternative (alleles) exist in a population. Studies of coding DNA to date have tended to focus on a few particular genes, possibly because they are believed to be functionally important and related to particular biological traits. Examples include the MHC, or growth hormone genes groups. However, overall, as yet relatively few gene sequences have been characterized for the aquatic species considered.

The characterization of variation in these regions, inlcuding the analysis of expressed sequence regions or tags (ESTs) in a number of commercially important aquatic species offers an important source of coding DNA sequences in the future. SNPs can be used for many purposes because they are very common (their frequency is estimated to be 1 each 1000 bp). These can be identified by producing and comparing nucleotide sequences of a given region for several individuals. Once the SNPs have been identified they can be typed easily by a number of methods from "easy" ones like restriction enzyme digestion, to "sophisticated" methods like DNA chips, real time PCR machines using TAQ-man probes, or molecular beacons. Their digital nature means that in the future SNPs will probably be the method of choice for monitoring animal and plant species to help define the interactions between natural and cultured populations.

Increasing genomic resources for many species, such as cDNA libraries, EST databases, and DNA microarrays are having a profound impact on research in areas such as agriculture and medicine. Since DNA microarrays can provide expression information for thousands of genes simultaneously, they are now the principal tools for conducting functional genomics research. The fabrication of cDNA microarrays involves gene/clone selection, PCR amplification of transcript sequences using universal primers, purification of PCR products, and robotic printing of PCR products (cDNAs) onto glass slides (6). cDNA or oligonucleotide microarray platforms have been built for several aquaculture species, including Atlantic salmon and common carp. Since microarray-based experiments have identified fish genes potentially contributing to fitness-relevant traits, such as precocious ovary development and

rapid growth, they may be useful in future research aimed at evaluating the impact of escaped aquaculture fish on wild fish populations.

In conclusion, identification of aquaculture escapees and their offspring in the wild may require different markers depending on species and situations. Some factors are decisive regarding how to develop / apply a genetic marker for a given situation; these include the genetic distance between domestic stocks and wild populations, the number of generations after accidental escapes or deliberate introductions occur, the genome structure of a species (with more or less multi-allelic loci). Practical feasibility (cost, equipment, technical difficulty, expertise required) is another aspect which needs to be taken into account, as well as the number of samples and sample sizes (one single episode of escapes? routine surveys?) and finally statistical analysis. When absolute markers exist for differentiating aquaculture escapees and native individuals, statistical analysis does not pose particular problems because immigrants and their offspring can be directly identified. However absolute markers (stock-specific) are rarely found within species, particularly for those with high dispersal capacity, as is the case of many marine animals. Fortunately, many statistical methods, implemented in readily available computer programs, are available which make it possible to detect immigrants in such cases with a high level of probability (7).

Main conclusions- Future research priorities

A number of conclusions can be reached based on the available tools for each of the studied species, (Table 1) as well as the various discussions that followed. These are:

- Several different types of genetic markers are currently available for monitoring cultured and wild fish, each with its own advantages and disadvantages and different genetical features (dominant/codominant, nuclear/mitochondrial DNA, maternal/ biparental mode of inheritance, number of loci detected per assay, number of alleles detected per locus) and different types of analytical procedures are required in each case with different technical and expertise requirements.
- The main markers developed in the studied species and which are currently used involve allozymes, PCR RFLPs (nuclear and mtDNA), RAPDs, AFLPs and microsatellites. The choice of marker in a given context depends on a number of factors including the specific question to be addressed (e.g. population structure, levels of genetic diversity, measuring differential fitness between farmed and wild fish, identification of farmed and wild stock) and the species under investigation (i.e. there is great variance on the amount of information available, in addition to technical and logistical considerations). Therefore, molecular markers should be assessed on a case by case basis. Some generalizations can be made, such as that microsatellites have more power to detect subtle differences, but other, older markers such as allozymes may still be of use in certain situations (they have proven to be valuable genetic tags in cod and in brown trout).
- In many situations, microsatellites will be the marker of choice in attempts to detect cultured individuals in the wild. Success in doing so will depend on having baseline data which can be used to assign the origin of individuals being tested, especially where makers diagnostic for farmed individuals are lacking as is the case in most situations. In such cases even the use of numerous, highly polymorphic loci cannot guarantee success, particularly where differentiation between cultured and wild fish is low. The possibility to detect escapees can be best assessed if there is a detailed knowledge of population structure of wild as

well as farmed populations for the markers considered.

- Information on population structuring is limited or lacking for most of the species of interest and research in this area should be a major priority in areas of aquaculture and situations where stocking is undertaken. Research should include extended spatial as well as temporal monitoring of populations, to ascertain if spatial structuring found is stable across space and time; a single set of samples from one time period may or may not reveal the true situation.
- Despite numerous works on various species with various molecular markers, existing research, data analysis and comparative studies on each species cannot easily be exploited due to lack of standardization among studies and marker characterization (sampling design, appropriate use of controls, replicate screening within and between labs). Intercalibration of results of different laboratories is still minimal. Databases of produced genotypes and of the genetic material of control individuals are still needed in most cases in order for European Science to take advantage of the previous efforts that have been spent on the genetic analyses of species of aquaculture interest.
- Communication with aquaculture stakeholders is also essential in order to be able to record and monitor information on the origin and number of broodstock for every species.
- A particular concern as regards data base integration is how to minimize genotyping errors (which have often been proven to significantly affect results) as well as how to maximize the information obtained from different statistical analyses. More work is needed to assess the statistical properties of the different theoretical models used and more research should be done with real data sets.
- Their distinct advantages mean that in the future SNPs and microarrays are likely to see increasing use. Much work is still needed for identification of SNPs and construction of microarrays. Genomic programmes are in progress or in the process of being initiated in most aquaculture species of interest, but studies of association between specific phenotypes (domestication) and possible linked QTL are still missing. This could help to identify markers for farmed fish. Priority should be given to identifying the genes involved in domestication, i.e. the changes in the genetic architecture of wild populations when brought under farming practices. This should allow the identification of the true functional differences of wild to farmed individuals and should therefore facilitate the identification of farmed escapees based on the genes that matter and not only with supposedly neutral markers. Where genomic resources are still not available for a species of interest, searching for functional polymorphisms and differentiation between wild and farmed individuals should focus on finding informative EST linked loci, which show differentiation between cultured and wild populations.
- More immediate alternatives are to consider the development of new genetically tagged farmed strains where possible, especially for ranching studies. In cases where extended breeding programmes have already started, like salmon, it will be difficult, but the possibility of selecting for a few molecular markers, to provide diagnostic variation, as a required part of selective breeding programmes should be examined for other species.
- The use of non-genetic tags and other strategies e.g. triploids, sterile fish to reduce impact of escapees should also be considered when reviewing monitoring technology options.

Species	Allozymes	Mitochondrial DNA	Micros	AFLPs	SNPs	Genomics	Other
Common carp *Cyprinus carpio*	19 variable loci of 60	D-loop, Cytb, ND3/4, ND5/6	>100	One study	PCR RFLPs	In progress	RAPD, SSCP
Atlantic salmon *Salmo salar*	33 variable loci of 110	D-loop, Cytb, ND1, ND5/6 >20 RFLPs >40 SNPS	~1700	Yes	Yes	Yes, well underway	Blood proteins
European sea bass *Dicentrarchus labrax*	>25	D-loop, Cytb	>250	> 200	In progress	In progress	Linkage map
Gilthead sea bream *Sparus aurata*	21-27	Dloop	127	147	Yes	In progress	
Turbot *Scophthalmus maximus*	>25	Dloop, Cytb, rRNAs, tRNAs	>35	No	No	In progress	
Atlantic cod *Gadus morhua*	>30	Whole	>80	No	>90 ; more in progress	>20 000 ESTs ; more in progress	
Atlantic halibut *Hippoglossus hippoglossus*	>40	No	>25	No	No	In progress	
European flat oyster *Ostrea edulis* *Crassostrea gigas*	22 24	Cytb, rRNAs Cytb, 16S rRNA	24 >120	296 > 300	No >50	No In progress	Genetic map Genetic maps
European lobster *Hommarus gammarus*	>40	Whole	>50	No	No	No	
Mussels *Mytilus edulis* *M. galloprovincialis* *M. trossulus*	15	Whole	7	No	Yes (PCR-FFLP)	In progress	
Scallop *Pecten maximus*	13	Cytb, COI, ND1, rRNAs, tRNAs	9	No	In progress	In progress	

Table 1. *Availability of different molecular markers in the Genimpact species*

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Tools for monitoring fitness of aquaculture individuals in the wild

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Introduction and objectives

The current danger from genetic impact of aquaculture activities has aroused a great deal of concern among scientists and the general public. The physical structures of aquatic culture are such that caged fish can escape and sessile species such mussels can disseminate eggs and sperm through water and therefore interbreed with wild conspecifics in the natural environment. Natural populations of many aquaculture species (for example salmon and cod) are localised and genetically differentiated. Many of these populations are at historical lows or close to endangered levels (1, 2). Protecting the genetic integrity of local populations and minimising the risk of fitness depression in wild populations should therefore be a focus of management and policy.

Fitness is defined as the relative reproductive ability of a genotype, and the ability of an individual to pass on genes to the next generation. Fitness is therefore dependent on survival and reproduction of the individual. Common garden field studies have demonstrated that escapees can survive and spawn successfully, contribute genetic material in the F_1 generation, and that such gene flow can reduce the fitness of wild stocks (3).

The main objective of this work package is to review and evaluate various methods used to measure fitness and monitor the effects of deliberate and inadvertent introductions on the characters and abundance of wild populations. Most studies on interactions between wild and cultured stocks at fitness level have been carried out on Atlantic salmon *Salmo salar* and cod *Gadus morhua*, thus this review will be principally focused on these two species.

Genetic diversity and fitness traits

The relationship between fitness and genetic variability has stimulated considerable interest (4, 5, 6) particularly in salmonid fishes. Although the generality of the relationship remains controversial (see below), a positive correlation between genetic variability and fitness is often observed (reviewed by 5, 6, 7). Positive associations have been observed between protein heterozygosity as assessed by molecular markers such as allozymes or microsatellites, and several fitness-related traits such as growth, metabolic efficiency, body size, fecundity and survival (6). A number of features have been proposed to contribute to this relationship (7). Briefly, mating between relatives increases the proportion of homozygous loci in offspring, therefore increasing the probability of recessive deleterious alleles occurring in a homozygous state, and therefore resulting in decreased fitness (8).

The importance of preserving genetic diversity has been emphasized for maintaining the long-term evolutionary potential of populations or species (9, 10). In one study, experimental groups of salmon fry harbouring unusually high or low levels of genetic variation were created, and it was then examined whether a behavioural trait important for fitness (aggression) differed between the groups (11). In a second study, salmon and trout stocks were partitioned into groups with either extremely low fitness (presence of a severe morphological deformity) or assumedly normal fitness (no obvious deformities) and then the levels of genetic variation in the two groups were compared (12). The results of both studies suggest that low levels of genetic variation may negatively affect the fitness of individuals in these populations, most likely due to inbreeding depression as a result of the use of low numbers of founding individuals in hatchery-reared populations. This highlights the advantages of the captive environment for studying heterozygosity-fitness correlations. Nevertheless, an important direction for future research would be to ascertain whether such genetic diversity-fitness associations are also observed in more natural conditions, preferably allowing an evaluation of offspring survival to reproduction.

MHC genes

Until recently, "neutral" nuclear or mitochondrial DNA loci have been the focus of population genetics in fish and other vertebrates. In this context, several statistical tests for neutrality were developed. Neutral loci were extensively used to investigate interactions between natural populations and conspecific reared strains, particularly of salmonid fish (3). Ferguson (13) first suggested that, where population structure had been established using neutral loci, adaptive gene loci may be informative. In the case of fish interaction studies, the major histocompatibility (MHC) genes have been particularly targeted.

Pathogen-driven balancing selection, with overdominance or heterozygote advantage, is believed to underpin high levels of polymorphism observed in MHC loci. *UBA* alleles also demonstrate considerable trans-species polymorphism, a good indicator of balancing selection. Challenge experiments in which domesticated salmonid stocks were exposed to a number of pathogens (14, 15) uncovered differential survival rates mediated primarily by MHC heterozygosity and/or overdominant selection. In many cases (16, 17) , it appears that variation at MHC loci may be a feature of local adaptation, as well as influencing survival in native trout when challenged by diseases carried by reared salmon. Therefore, reared salmon may negatively impact on wild salmonid populations, putatively via disease transmission, in addition to having direct and other indirect genetic (3).

QTL

Quantitative traits are traits with measurable phenotypic variation usually influenced by multiple polymorphic genes and environmental factors. A quantitative trait locus (QTL) is a region of DNA associated with this trait. QTLs are not necessarily genes themselves, they could simply be stretches of DNA closely linked to the functional polymorphisms that underlie the trait in question. Examples of quantitative traits range from agricultural yield to disease resistance, to a variety of evolutionary relevant traits including growth rate, reproductive output, and even fitness (18).

In addition to QTL mapping, several other strategies exist for identification of functionally important variation, including multiple-marker-based 'neutrality' tests

(also known as hitchhiking mapping and genome scans), environmental association studies, admixture mapping and association analysis (reviewed by 19). It is likely that by combining QTL analysis with such complementary analysis strategies increases our understanding of the genetic basis of phenotypic diversity and provides new insights to the evolutionary processes behind adaptation and speciation. Recent QTL studies have also begun to explore increasingly sophisticated issues such as genetic correlations, gene-by-environment interactions, epistasis and the adaptive importance of particular genes. Altogether, these advances should also permit the analysis of QTLs directly related to fitness of aquaculture individuals in the wild, and/or the changes in the genetic architecture of wild individuals being domesticated.

Monitoring fitness of individuals of different origin in the wild

Most farmed fish are intensively farmed in sea-pens in sheltered areas. Experience with salmon has shown that it is extremely difficult to stop accidental escapes and recent research indicates that other species like cod are even more likely to escape sea-pens. During the 1990's, a number of studies were conducted to compare the spawning success of escapees and wild salmon (20). In general, the spawning success of escaped salmon was found to be lower than that of wild salmon, and particularly male escapees were performing poorly, while the females appeared to be more accepted. In spawning areas with a high abundance of wild spawners, the spawning success of escapees tended to be very low. Studies on behaviour of escaped farmed salmon during the first days and weeks post escapement have also shown that due to low survival and little migration of farmed fish, they are not likely to migrate into spawning areas of wild salmon (21).

Less is known about the survival and fitness of farmed salmon that escape at the smolt stage. There are indications that these may pick up a migration pattern more similar to wild salmon, particularly if they escape during spring. It is likely that a higher proportion of these escapees will find their way to spawning areas of wild salmon, and have a higher survival and spawning success than farm salmon escaping at later life stages. This is an area where more data are needed, in order to recommend and give preference to measures to reduce genetic impact on wild salmon.

Animals express very different levels of performance under laboratory compared to field conditions, as experiments undertaken in laboratory environments (e.g. hatchery tanks) are unlikely to mimic the stressors encountered in natural environments such as pathogens, predators, competitors and physical stress (22). DNA profiling now enables the identification of an individual's parentage providing a direct comparison, under common environmental conditions, i.e. in the wild, from the egg stage onwards, of traits related to fitness. Marker development and access to increasingly sophisticated analytical technology with complementary increases in throughput and speed of analyses provides significant opportunities in terms of experimental design for field experiments.

Having released groups of fish into the wild it is important to be able to recover individuals, the survivors and if possible the mortalities (e.g. determine causes of death), to assess population numerical strength, determine survivorship (mortality), to make measurements e.g. weight, length and condition, to determine behavioural patterns e.g. migrations out of the experimental area. It is important to be able to collect sufficient numbers of samples to ensure statistical rigour. The mortality and survival experience of a whole population, over an entire life cycle, can be evaluated in a life table, and within which the relative 'force of mortality' acting [sic] at a specific

life history stages can be isolated and assessed. Critically information organised in life tables can used as the basis to derive other important fitness metrics such as the net reproductive rate and intrinsic rate of increase.

It is important to be able to make observations on mortality and deformities rates during egg development. Egg development can be easily monitored in the hatchery. In the river, artificial salmon redds provide an excellent opportunity to observe egg development success under natural conditions. Also the hatchery offers good opportunity to monitor what is going on in the wild as a control.

Future Research Priorities

- Identify and develop molecular markers to identify introgressed individuals.
- Evaluate the extent of introgression of cultured individuals in natural populations.
- Study the consequences of introgression by using common garden experiments or other appropriate methods.
- Search for major common genes (QTL mapping, microarrays) affected during domestication.
- Use modelling and molecular approaches to study the genetic architecture of the domestication and selective breeding.
- Develop phenotypic and molecular correlates for use as predictors of fitness and consequence of genetically altered organisms (selected or genetically engineered) in nature.
- Expand studies of MHC and other fitness related single and multi-gene traits in cultured species.
- Explore spawning behaviour and interaction between farmed and wild spawning fish and shellfish.
- Develop methods to identify individual performance among wild individuals in comparison to farmed ones.
- Estimate absolute size of native spawning populations in relation to number of escapees or cultured individuals in the wild.
- Study dispersal and migration of escapes (horizontal and vertical).
- Set up common garden fitness experiments for species lacking information.
- Ecological complexity of models should be increased to incorporate density dependants effects.
- Evaluate fitness consequences of transfer of strains and exotic shellfish species of aquaculture.
- Evaluate the consequence of replacement of natural populations by individuals from hatcheries (transferred) fish and shellfish species.
- Evaluate and model changes in fitness of specific farmed strains among ecosystems.
- Identify causative polymorphisms (e.g. indels, SNP) that underlie genetic basis of fitness traits.

Conclusion

To monitor fitness of farmed individuals as well as changes in fitness of wild populations due to introgression, it is critical to have knowledge of the biology of the species being studied particularly of the key life history events such as maturity schedules, growth and reproduction strategies. Equally, a prior knowledge of the genetic constitution of the contributing broodstocks as well as the wild stocks is a prerequisite. When studying fitness of wild and farmed stocks, the opportunity to produce genetically informative matings to provide information on hybrids, half sibs, dam effects, sire effects, molecular variation, QTLs, quantitative traits should be taken. The applicability of common garden experiments will depend on potential for containment. In low containment experiments there will be a need to be able to screen out individuals not introduced into the wild as part of the study. This includes individuals from other cohorts, the same cohort, resident, but not introduced as part of the experiment and therefore of unknown parentage and migrants into the experiment.

The screening out process will require rapid and high throughput analytical capacity. Experiments are required that ideally will provide global and multigenerational measures of lifetime reproductive fitness. It will not always be possible to undertake such broad scale experiments for logistical reasons. Local or stage specific measures of fitness can be considered but caution is required in the interpretation of the results obtained. In order to assess fitness at local or global levels there must be the capacity to recover sufficient samples to provide for robust statistical analyses.

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III

Predictive tools. The use of modelling to assess the risk of genetic impacts on wild populations from escapes of cultured fish

Workshop 3 brought together a range of leading experts for 3 days to consider how modelling could advance understanding of the potential genetic impacts of escaped cultured fish on wild populations. It reviewed existing modelling work, assessed different modelling approaches which might be used, considered their strengths and weaknesses in informing on the issue of impacts, and identified research priorities. The output of the workshop has been summarised in a scientific paper. The basic issues and conclusions of this paper are summarised in the three chapters contained in this section.

Participants to the Pitlochry (Scotland, UK) workhop, 15th – 17th February 2007

Front row (from left): *D. Crosetti, T. Svåsand, E. Verspoor, K. Glover, J. Gilbey*

Back row (from left): *P. McGinnity, R.H. Devlin, O. Diserud, P. Bacon, T. Cross, J. Tufto, C. Thompson, B. Gjerde*

Not in the picture: *N. Barton, J-P. Vähä*

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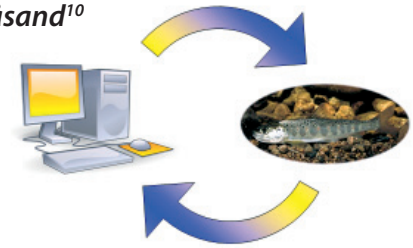

Genetic interactions between cultured and wild stocks: the problems

Recent years have seen significant increases in the European aquaculture production of fish and shellfish species such as: Atlantic cod, Atlantic halibut, Atlantic salmon, common carp, European sea bass, gilthead sea bream, turbot, European lobster, scallops, mussels, and oysters. Concurrent with these increases comes concerns as to the potential genetic impact on wild populations of escapes and of cultured individuals (1).

Cultured individuals typically represent genetically exogenous populations, and in addition, the genetic constitution of culture populations has frequently been altered through inbreeding, selective breeding, or domestication. These differences become more pronounced the longer a species has been in mariculture production, particularly when closed life-cycle production is used (i.e. using cultured stock as broodstock), but can become apparent even in the first year of production (2). Interbreeding of escaped cultured stocks with wild populations can have serious consequences for the fitness and long-term persistence of the wild population. Introgression of non-native genotypes into wild populations can result in the break-up of local adaptation, resulting in, sometimes dramatic, reductions in fitness of these stocks (3) (Fig.1).

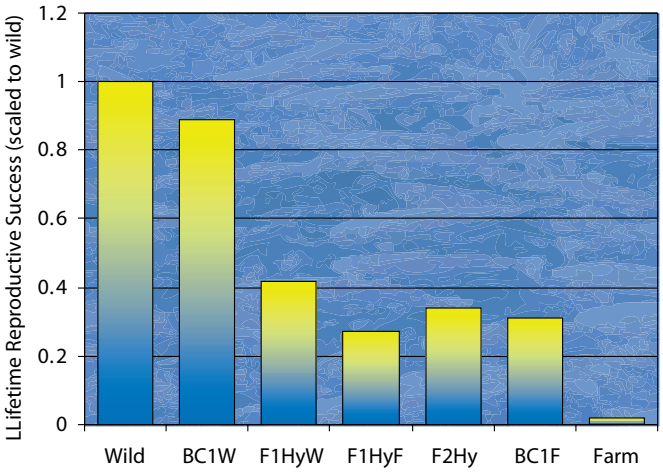

Fig. 1. *Example of fitness differences between wild, farmed and hybrid fish: lifetime reproductive success of Atlantic salmon (3). Cross types refer to: BC1W F1 hybrid x wild; F1HyW wild female x farm male; F1HyF farm female x wild male; F2Hy F1 hybrid x F1 hybrid; BC1F F1 hybrid x farm.*

Genetic introgression and fitness: the evidence

The whole range of outcomes, from no detectable effect to complete introgression or displacement has been observed after interactions of escaped cultured stocks and wild populations. Where they have been measured, such interactions always appear to have negative effects on the wild population (4) (Fig.2). These findings are in agreement with theoretical models which predict the breakdown of local adaptation during introgression of non-native genotypes.

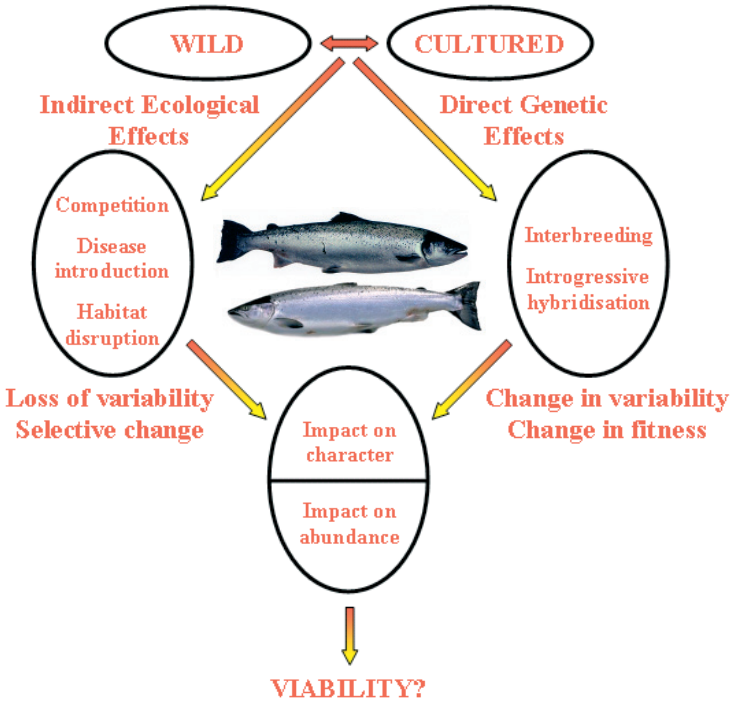

Fig. 2. *Genetic and ecological effects of interactions between escaped cultured fish and wild stocks*

Effects recorded include decreases in survival at various life-history stages, changes in growth rates and development, a reduction in fecundity, and ultimately, significant decreases in lifetime reproductive success. All these influences interact to reduce the fitness both of individuals and the population as a whole (5). 'Common garden' life-cycle experiments studying farmed and wild salmon, and their crosses, in natural environments have been carried out in Ireland and Norway and well illustrate the potential scale of the problem (3, 6). These studies have found that famed fish may exhibit a lifetime success of ~2% compared to the wild. Further, the lifetime success of various types of hybrids between these two stock types ranged from 27 to 89% of the wild (Fig. 1). It has been suggested that interactions of this type can result in lowered fitness in the population as a whole, with repeated escapes having the potential to cause cumulative fitness depression and potentially an extinction vortex in vulnerable populations (5).

Why use a modelling approach to study these problems?

The difficulty of predicting the effects of influxes of exogenous stocks on local populations has been shown to be consistent with population genetics theory (7).

Reliable predictions would require that all population parameters are known; but this never occurs in real situations; even basic parameters such as life-cycle descriptions and effective population sizes are often poorly understood. Further, the genetic basis for variation in most fitness traits, such as growth, development, maturation and fecundity, are also largely unknown. Thus there is often little knowledge of the extent to which most genes are subject to selective pressures, fitness differences and/or genetic drift.

The only available solution to this problem is to use the results of empirical studies to develop computer based simulation models. Empirical studies comparing the fitness of wild and escaped cultured stocks, whilst essential for providing baseline measurements of individual and population level fitness, are very expensive and time consuming to perform; this is particularly the case given the many native species now cultured in Europe. It is also difficult to extrapolate from the limited experimental studies to different mixing and local scenarios. For example, the common garden experiment carried out in Ireland on farmed and wild salmon, and hybrids, under natural conditions, although it resulted in invaluable data collection (3), took 10 years to complete yet only studied impacts over two generations of introgression for a single species and a single site.

In contrast, computer-based models of population mixing can easily be used to simulate ecological and genetical interactions between cultured and wild stocks for a complete range of conditions. Thus in principal, at least, modeling can provide a comprehensive insight into the potential consequences for population character and abundance. However, for the modeling to be informative, it needs to contain a realistic representation of both demographic and genetic processes and parameters (Fig.3).

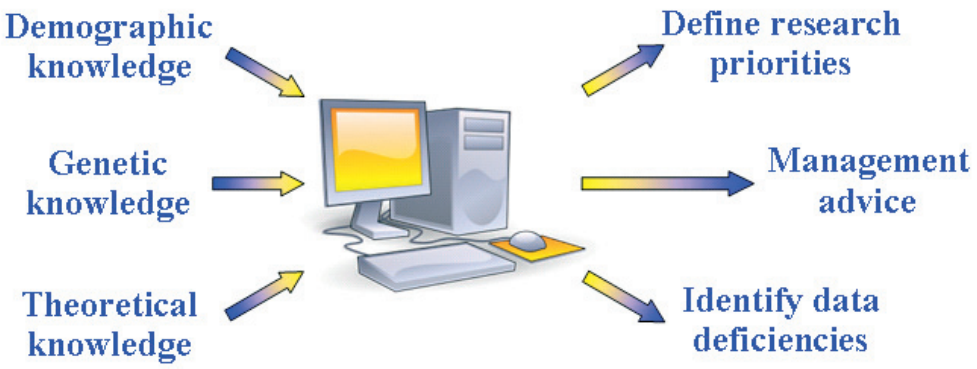

Fig. 3. *Modelling rationale*

A combined modelling approach allows different interaction scenarios to be evaluated for wild populations which will often differ in their demographic and genetic character. In doing so, both a broad-scale overview of expected interaction outcomes can be obtained as well as location-specific population impact predictions.

Just as importantly, modelling is invaluable in helping to identify where genetic or demographic processes are poorly understood or data is lacking. These deficiencies often already become apparent at start of building of such a model. This involves a detailed analysis of the scientific literature relating to species life-cycles, demographic and genetic processes and, in particular, the genetic basis of fitness related traits in

the species under investigation. Knowledge gaps also become apparent during model development and corroboration of the model using empirical data.

Thus combined modeling of cultured-wild interactions, which encompasses both demographic and genetic processes, provides:

- the best way of identifying the impacts from which might be expected both generally, and specifically in any given situation.
- an invaluable tool for identifying research needs and priorities.

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Modelling of impacts

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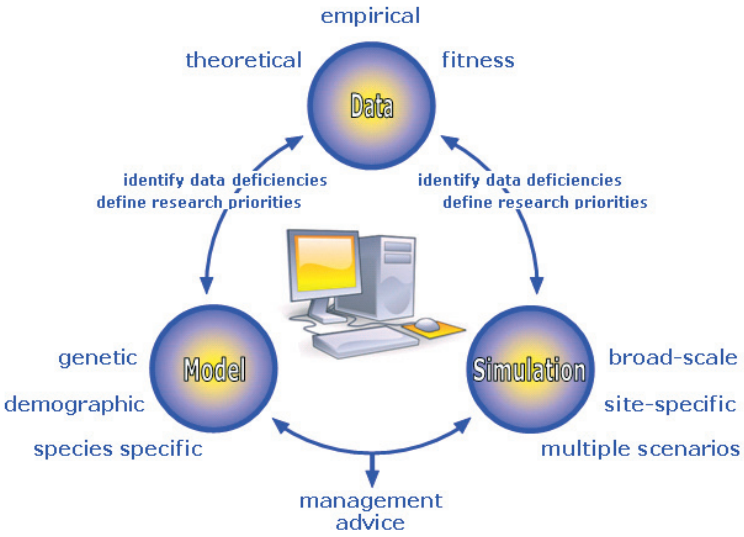

The modelling approach

Empirical studies comparing the fitness of wild and escaped farmed stock, whilst essential for providing baseline measurements of individual and population level fitness, are very expensive and time consuming to perform, particularly for the many species that are undergoing increases in production. It is also difficult to extrapolate from these studies to different mixing and population specific scenarios. Computer-based models of population mixing help overcome these problems. They can be used to simulate ecological and genetical interactions between farm and wild fish for a range of conditions, and provide insight into the potential consequences for population character and abundance. This can be achieved by models which include both demographic and genetic parameters. The basic modelling approach is outlined in Fig 1.

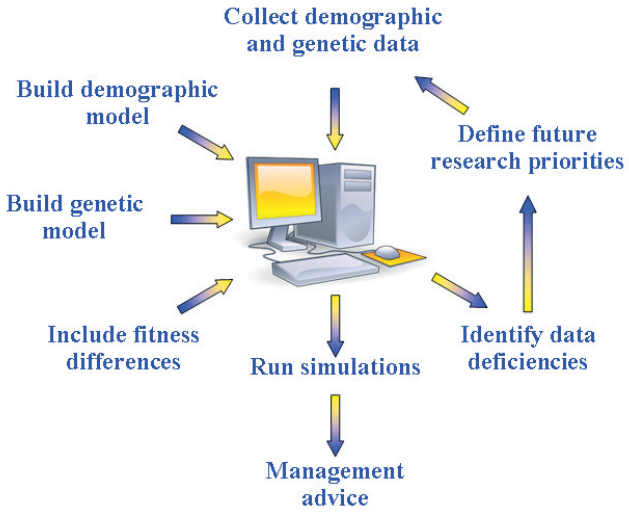

Fig. 1. *Basic steps in the development of a culture/wild interaction model.*

Existing modelling work

Finfish

To date, interaction models have been restricted to salmonids. Hutchings (1) modelled the threat to wild populations experiencing various intrusion rates of escaped farmed salmon and small or large fitness differences among the offspring. In Hindar *et al.* (2), fitness is presented by life-history stage in a fitness component analysis, and the range of scenarios narrowed down based on the whole-river experiments in Ireland and Norway (3, 4, 5). The model of Hindar *et al.,* focused on the effects of interbreeding and varying fitness parameters between different offspring groups (Wild x Wild, Hybrid, Farm x Farm), and how these parameters may affect the group proportions through time. The model consists of three main elements:

1. Intrusion. The annual intrusion rate of escaped farmed salmon into the spawning population. This rate can be assumed to be fixed, or it may vary from season to season according to time series observations or some stochastic process. Whether the farmed salmon escaped early or late are considered.
2. Spawning. The relative spawning successes of the different groups, including mature male parr, can be varied. Sex ratios, size differences etc. can also be considered in the model.
3. Survival. The offspring groups have varying relative survival rates for different life-history stages.

The basic simulation, with a fixed intrusion rate of 20 % escaped farmed salmon in the spawning population and average fitness parameters, suggests that substantial changes take place in wild salmon populations within ten salmon generation (~40 years). Low-invasion scenarios suggest that farmed offspring are unlikely to establish in the population, whereas high-invasion scenarios suggest that populations are eventually composed of hybrid and farmed descendants. It was concluded that further measures to reduce escapes of farmed salmon and their spawning in wild populations are urgently needed.

Transgenic fish

Transgenic fish strains have been in development since the mid 1980s, but as yet, none are known to have entered natural ecosystems. Nevertheless, a major objective of some transgenic fish research is development for aquaculture of strains with enhanced production characteristics such as improved growth rates and feed-conversion efficiencies (6). Significant concern has emerged regarding potential ecological effects of transgenic fish in the wild as well as approaches and uncertainties associated with risk assessment methodology (6, 7, 8). Developing accurate modelling approaches to estimate the consequences of transgenic fish in nature is therefore essential to assist with their safe application in the future and this is perhaps the best modelled area of possible cultured/wild genetic interactions.

Several deterministic models have been developed to explore variables which may influence the persistence of a transgene in fish populations (9, 10). These modelling exercises revealed that from a practical perspective, releasing transgenic individuals with multiple transgenic loci could significantly reduce the number of generations required to establish the transgene in 99% of individuals in a population, and that such effects are more pronounced when stocking densities are less than 40% of the natural population size. It was also found that the speed of transgene introgression could be underestimated if factors such as variable clutch size and survival to maturation were not considered, particularly in species with short life cycles.

Models have also examined the influence of fitness effects of transgenic fish (11). It was found that higher rates of introduction elevated transgene equilibrium frequency in populations when the transgene confers a deleterious effect. In cases where a hemizygote advantage exists, wild type fish persist in the population and the transgene reaches a maximum equilibrium frequency of 0.5 (lower in cases where fitness differences exist between homozygous transgenic and wild-type fish).

Models incorporating effects of multiple fitness parameters and life history characteristics on both transgene frequency and population size have also been developed (8, 12). A range of outcomes have been predicted using these models, depending on the interaction scenarios examined. Reductions in transgene viability or enhancement of male mating success could respectively eliminate or drive to fixation a transgene in populations, with minimal effects on population size. In contrast, combining negative and positive fitness values could have very profound effects on fixation and population size, perhaps even ultimately driving the population to extinction. This consequence has been termed the Trojan gene effect (for example see Fig.2).

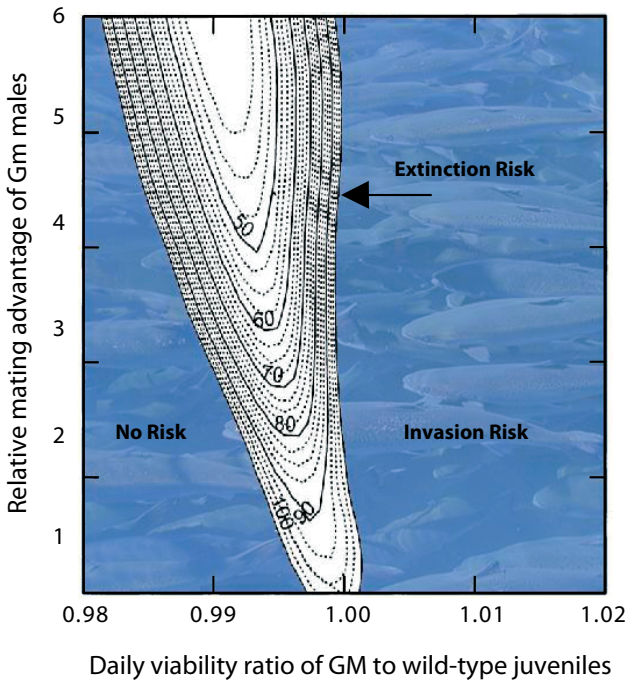

Fig. 2. *Predicted time to extinction in generations (contour lines) as a function of the ratio of GM to WT daily juvenile viability and GM male mating advantage relative to WT males (after 13).*

Shellfish

To our knowledge, there is currently no modelling study dealing with the risk of genetic impacts of cultured shellfish stocks on wild populations. Most existing modelling studies on cultured shellfish species focus on bivalve molluscs. They primarily deal with population interactions with the environment, and are used to assess the carrying capacity of a studied ecosystem (typically a bay, estuary or lagoon) in terms of aquaculture production, and to optimise rearing practices and management techniques (14, 15, 16). Most of these models are based on a rather precise description of the environment through the simulation of the spatio-

temporal dynamics of hydro-biological conditions. The environmental module is then coupled with a module simulating bivalve physiology and bioenergetics that determine individuals' life history traits and in turn their population dynamics (15, 17). Most of these modelling studies deal with monoculture but a few models account for polyculture (15, 17). The latter would be a good starting point for modelling the interaction between cultured stocks and wild populations since in mathematical terms modelling several populations is rather similar to modelling several species.

Development of new models: a framework following the development of an individual-based model of Atlantic salmon interactions

The development of interaction models for many of the species in aquaculture benefits greatly from the experience of pre-existing models. One such model is the recently developed individual-based model of cultured/wild interactions in the Atlantic salmon (18, 19). The steps used in the production of this model follow those outlined in Fig. 1 and provide a framework for building interaction models with other species. The novel genetic component of this model, linking genotype to phenotype over many generations, could also be of particular benefit in the examination of other species.

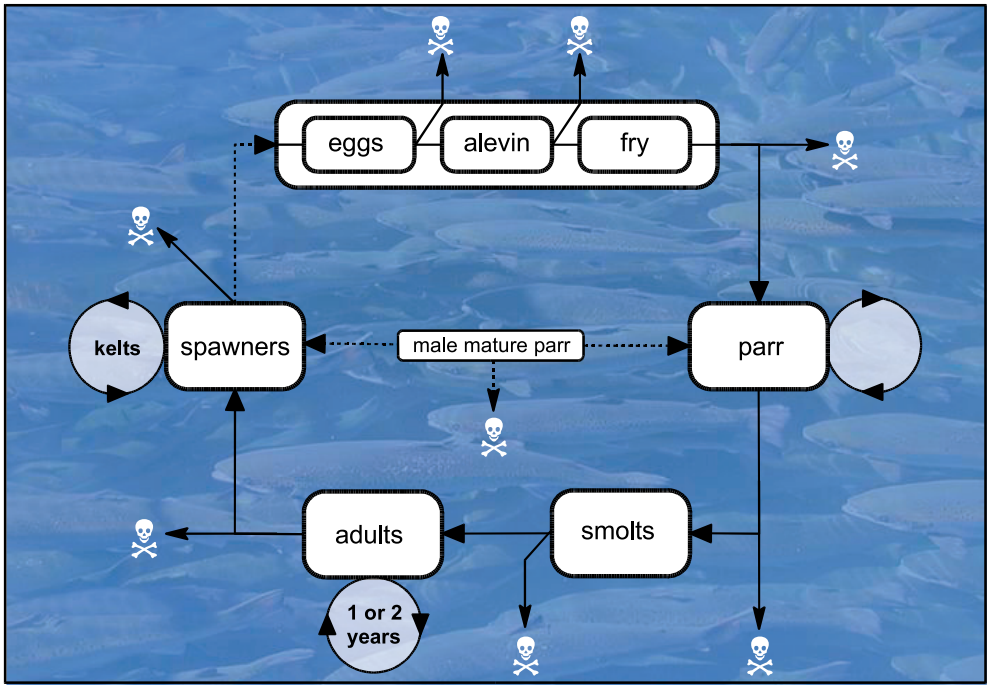

Fig. 3. *Demographic life-cycle model of an Atlantic salmon population.*

Demographic model

Data is collected from the literature detailing the life-history and demographic interactions of a wild individual/population. A life-cycle model is then built in the computer (Fig. 3), and parameterized using the information collected. The model follows individuals from egg, through the various developmental stages, until post-spawning death. As an individual moves between stages, growth and the probabilities of maturation and survival are calculated for each fish. These are based on a synthesis of population demographic parameters collected from the literature and weighted by the various individual trait values of a particular fish. The model is then

corroborated against empirical observations under a number of different scenarios to make sure it is capable of realistic representation of an unimpacted wild population. This stage gives an ideal opportunity to systematically examine the availability of data describing the various parameters involved, and thus to identify any gaps and define research priorities. During the development of the salmon model (18) the need for further information on the various density dependent processes acting in the freshwater juvenile phase was identified, and research projects were begun to examine these factors.

Fitness differences

In order to model the influence of escaped cultured stocks on the wild population, the demographical, behavioral and fitness differences between wild and escaped cultured individuals must be collected/determined. Again, this procedure allows the identification of knowledge gaps, and the defining of research priorities. Monitoring in wild scenarios is one tool to examine these influences, but of particular use here is the use of 'common-garden' experiments. These are experiments where mixing scenarios are initiated in the wild, and the population/s monitored over multiple generations, with the measurement of many individual and population parameters. During the development of the salmon model invaluable data was collected from the common-garden experiments carried out in both Ireland (3, 4) and Norway (5) (see Fig.4), and similar data must be collected for parameterisation of models for other species.

Trait	Gene complex/ fitness influence	Scaled to wild			Fitness Differential
		Wild	F_1	Farmed	
Egg survival to hatch (%)	embryonic	1		0.333	-66.67
Alevin size at first feed (mg)	embryonic	1		0.888	-11.22
0+ size (mm)	embryonic	1	1.146	1.171	+17.05
1+ size (mm)	freshwater	1	1.174	1.145	+14.52
Egg to smolt survival (%)	freshwater	1	0.615	0.410	-59.00
Smolt age	freshwater	1	0.798	0.637	-36.31
Mature male parr at 0+ (%)	freshwater	1	0.722	0.778	-22.22
Mature male parr at 1+ (%)	freshwater	1	0.667	0.232	-76.81
Relative male parr reproductive success	freshwater	1	2.237	3.953	+295.26
Smolt to adult survival (%)	marine/adult	1	0.850	0.333	-66.67
1SW length (mm)	marine/adult	1		1.127	+12.70
Sea age (% 1SW)	marine/adult	1		0.141	-85.86
Relative mature male reproductive success	marine/adult	1		0.2	-80.00
Fecundity (egg no)[4]	marine/adult	1		1.290	+29.04
Egg size (mm)	marine/adult	1		0.972	-2.76

Fig. 4. *Fitness differentials associated with various life-history stages of Atlantic salmon based on published empirical measurements. Population measurements are compared to a wild type scaled to 1. Fitness Differential is the percentage difference in farmed compared to wild fish (for references see 19).*

Genetic model

The underlying genetic architecture and gene/allele interactions of quantitative fitness traits are often extremely complex, and include additive, dominant, and epistatic influences (20). Although there is little information as to the relative importance of each of these influences, empirical observations suggest that a large proportion of the genetic basis for fitness in salmon can be described by a simple additive model (4) and this additive genetic model was therefore used in the novel genetic component of the salmon interaction model (19).

The second component of the genetic architecture that must be modelled is the distribution of genetic effects across the number of loci influencing the quantitative trait. These effects were modelled using an exponentially declining effects distribution model (21). Hayes and Goddard (22) carried out a meta-analysis of QTL mapping studies in livestock to infer the distribution of the effects of genes. They found the distribution of QTL effects was moderately leptokurtic, with many QTL of small effects and a few of large effect, consistent with the exponentially declining theoretical model of QTL influences. Similar evidence for the exponentially declining effects model has also been seen in salmonids (23).

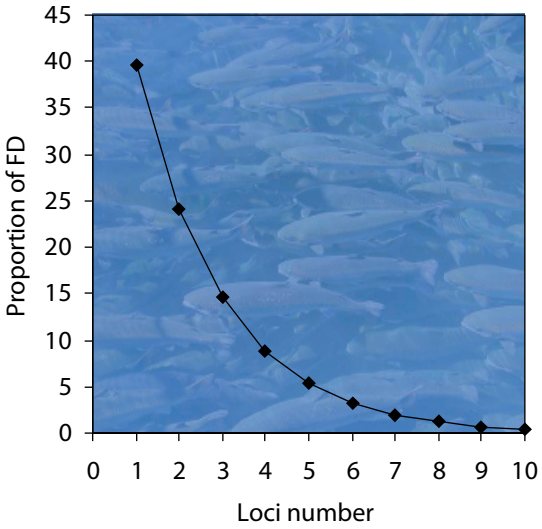

Fig. 5. *Distribution of Fitness Differentials across loci (total FD sums to 100%).*

The basic genetic model used was based on three sets of 10 unlinked loci, having an exponential distribution of Fitness Differentials (FD; i.e. the difference between a wild and cultured fish) (Fig. 5). Each set of 10 loci influences the fish in one of the three main life-history stages; embryonic, freshwater or marine/adult (see Fig. 4). The influence of genotype on phenotype is based on the interaction of empirically measured FDs and individual genotypes. Pure wild fish are defined as w/w homozygotes at all loci. Pure farmed fish are defined as f/f homozygotes at all loci, and it is these f alleles which are associated with the FD's calculated at each loci. Pure wild fish do not possess any f alleles, their total FD will thus sum to 0, and therefore they do not experience any change in the various population parameters (growth, survival, fecundity etc) associated with non-native inputs. Thus, the population parameters associated with pure wild fish remain those in the original wild population model. Pure farmed fish are f/f at all loci, and therefore the total sum of FD's over all loci for these fish will be 100. Individual fish with this genotype will therefore experience 100% of the empirically measured changes to the

various population parameters that have been found to be associated with farmed fish compared to wild.

Hybrid fish will have their total FD's calculated in the same way. F1 fish are w/f heterozygotes at all loci. Their total FD will therefore be the sum of single allele effects (i.e. ½ of the full loci effect) at each loci. For these cross types this will sum to 50 and these fish will thus experience 50% of the FD associated with the various population parameters. Other hybrids will have varying proportions of f alleles. Each f allele will have a FD defined as ½ the total effect at a particular loci, and the total FD for a fish will be the sum of each of these individual allelic effects. This, in turn, will define the proportion of the empirically measured changes to the various population parameters defined by the FD's that will influence the individual fish.

This novel genetic model has been developed to avoid some of the problems associated with previous 'mixing-models', where modeled behavior is based on the 'percentage hybridity' of an individual. Theoretical and empirical investigations (21, 22) have shown that the distribution of effects of fitness traits follows an exponential pattern. Thus, a 50% hybrid fish (F1) will experience significantly different fitness effects depending on the actual alleles it possesses, rather than just the hybrid generation it originates from. This basic model could be included in any future interaction models of new species, and as development has already been undertaken, could save significant time in the production of such models, whilst at the same time being more realistic than current genetic components.

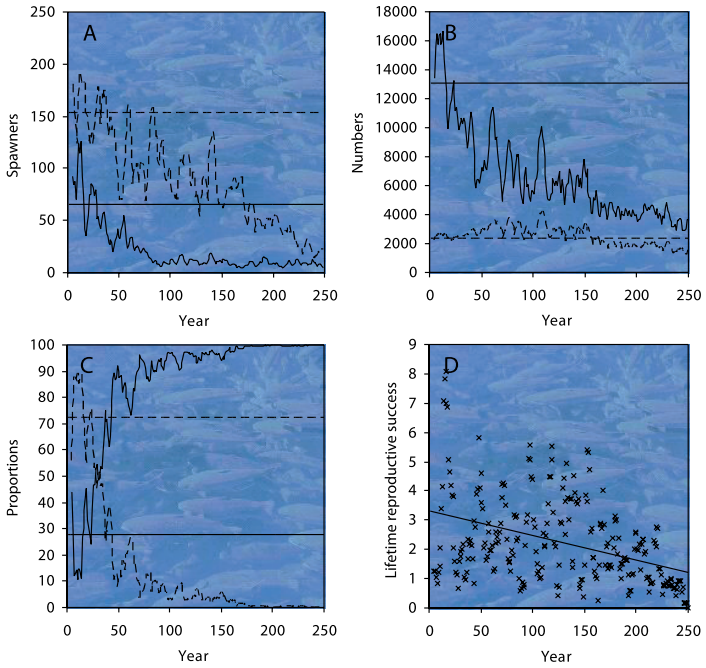

Fig. 6. Example salmon model partial outputs (19). A) Comparison of adult spawner numbers with 50 ± 5 non-native inputs per year into the wild population at mean ocean survival of 6% (dashed line) and 3% (solid line). Horizontal lines represent mean modelled spawner numbers with no non-native inputs at 6% (dashed line) and 3% (solid line). B) yearly parr (solid line) and smolt (dashed line) numbers. Horizontal lines represent mean modelled parr (solid line) and smolt (dashed line) numbers with no non-native inputs. C) proportions of 3+ (solid line) and 2+ smolts (dashed line). Horizontal lines represent mean modelled smolt proportions, with no non-native inputs, of 3+ smolts (dashed line) and 2+ smolts (solid line). D) Lifetime reproductive success of female spawners excluding that years non-native inputs. Note, all measures are 5 year running means and B, C and D are at a mean ocean survival of 3%.

Framework for developing modelling of cultured-wild interactions

Modelling studies performed with the Atlantic salmon provide the basis for identifying a basic systematic approach to the development of interaction models for other species. In addition to the need to encompass both demographic and genetic processes, the approach involves the following basic steps:

1) collecting from literature life-history, demographic and genetic information on key processes and parameters, including data on demographical, behavioral, and fitness differences between wild and escaped cultured individuals

2) building basic conceptual model including both demographic and genetic components

3) programming integrated demographic and genetic model

4) setting of realistic demographic, genetic and fitness parameters model

5) corroborating demographic and genetic components of the model by comparing outputs with empirical data and assessing effect of different model settings and structures

6) running of simulations across range of interaction situations, or for specific scenarios of interest.

Taking a systematic approach to the development of interaction models ensures knowledge gaps to be identified at each stage. Corroboration of the model is essential to give confidence in model predictions so that results of simulations of interaction scenarios provide a scientifically supportable basis for management decisions to be made. Using the modeling approach, information from existing empirical studies can be generalized, and the need for more, expensive and time consuming experiments minimized, and future research priorities defined more clearly and precisely.

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Research priorities in modelling

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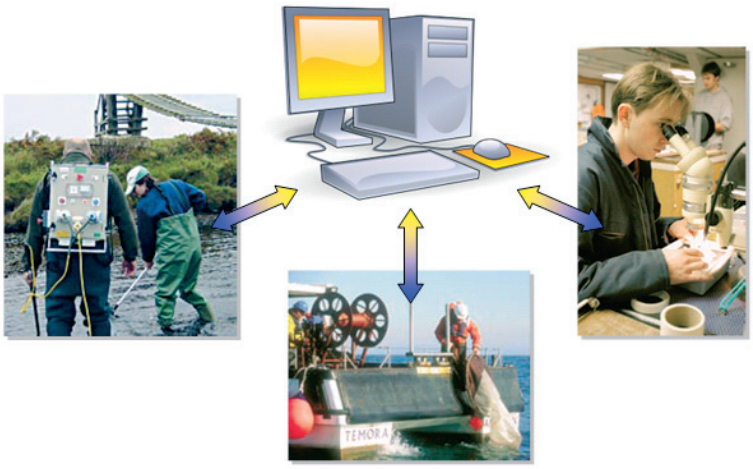

The modelling approach

Population models can be invaluable tools for extrapolating from limited empirical observations to allow examination of different cultured/wild mixing scenarios and site specific observations, ranking different management scenarios, and assessing the risk of extinction and conservation status of a target species. The modelling process involves specifying a set of rules based on the life history characteristics of the species, that determine how the number and character of individuals within the population changes over time. Both data availability and data quality can be limiting factors during model development, influencing the estimation of parameters and the understanding of demographic processes. In such circumstances, uncertainty potentially arises from a number of sources, influencing model structure, parameter estimation, and ultimately, model outputs and management advice (1). The building of computer based population models requires a detailed analysis of the scientific literature relating to the life-cycles, demographic interactions and genetic basis of fitness related traits in the species under investigation. It thus also provides an invaluable tool for the identification of data deficiencies, and hence identifying areas of future research priority.

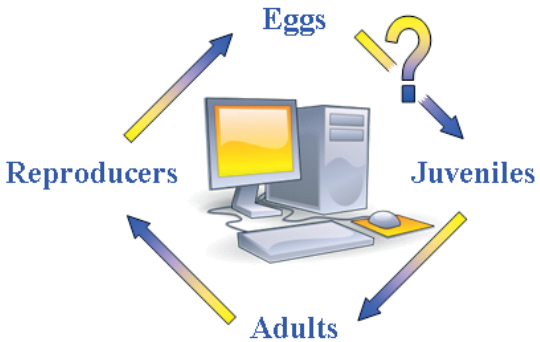

Fig. 1. *Describe life-cycle, collect demographic data, identify knowledge gaps, and define research priorities.*

Demographic processes

Basic population demography

The preeminent requirement in the development of a useful model, individual or processes based, is an understanding of the life-cycle and demographic processes of the species under investigation (1). Successful population models *must* be based on realistic demographic parameters. For some cultured species, many of these wild population parameters have been extensively studied over many years (e.g. Atlantic salmon), although information may still be lacking (e.g. density dependent processes in juvenile Atlantic salmon) (2). However, for others, even basic knowledge of life history parameters is limited. The first research priority must thus be the identification of areas for which data is lacking. Life-cycles of species of interest must be described, and demographic data collected from the literature. This processes will allow areas of deficiency to be identified and research programs to be developed to address these issues, and so allow successful model parameterization.

Fig. 2. *Farmed (top) and wild (bottom) Atlantic salmon.*

Differences between cultured and wild individuals

Together with the requirement to describe the basic wild population, comes a need to identify demographical, behavioural and fitness differences between wild and escaped cultured individuals. Ecological and behavioural interactions between wild and farmed individuals are complex and variable. Selection due to domestication has been shown to result in behavioural and morphological changes among the reared populations. In general, hatchery reared stocks have been shown to exhibit less dispersal range, less homing ability, lower predator avoidance, greater aggressive behaviour, less reproductive success due to lower fecundities, lower fertilization success or incompatible spawning behaviour (3, 4, 5, 6). Common garden experiments in the wild have also shown fitness differences in traits such as growth rate and development (7). However, information on demographical, behavioural and fitness differences among escapees and wild fish are for most species either completely lacking or severely limited. The majority of research has been conducted on salmonids. Consequently, much more information are needed before we can make reliable predications about the risk of hybridisation between farmed and wild stocks in other fish species. This observation is even more true of shellfish species. If little data is available on fish, data on fitness differences in shellfish is even more limited. Investigations have been restricted to examination of competition and hybridisation scenarios between cultured and wild stocks and also into the potential for local adaptation after translocations (8, 9, 10, 11). The second research priority is, therefore, to examine behavioural, demographic and ultimately fitness differences between wild and escaped cultured stocks of all species of interest.

Genetic processes underlying fitness differences

Once fitness differences have been identified, they must be included in the population models. This requires an understanding of the genetic architecture and genetic processes underlying such fitness traits. Such information is, and has been, the focus of much scientific investigation over many decades (12, 13, 14). The state of the knowledge is such that it is now possible for modellers to be able to develop realistic genetic model components. However, while these models are of great use in the investigation of cultured/wild interactions, and should be included in any realistic model, there is still much lacking information. The third research priority is therefore to increase our understanding of the genetic processes underlying fitness traits. This can be achieved through investigations in the wild and laboratory using both the species of interest, and model species. The use of model species is of particular use here, due to the, often long, generation times, expense and difficulty of maintenance of using the actual species to be modelled.

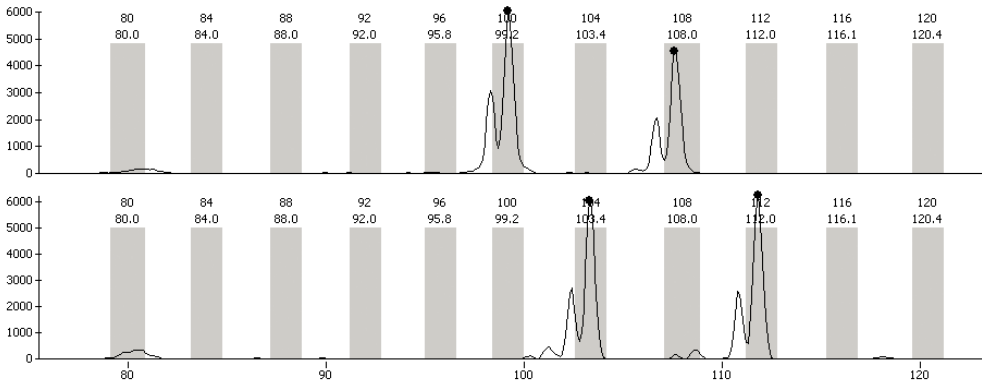

Fig. 3. *Single microsatellite genotype of two farmed cod.*

Identification of escaped individuals

Identification of escaped individuals in the wild is essential in order to understand the scale of the potential problem, and thus provide realistic scenarios for modelling. Identification of escapees is assured if all farmed fish are physically tagged, however tagging may not always be a realistic option due to high cost or biological restrictions such as the size of the cultured individuals. Genetic markers are commonly employed instead (15, Fig.3). They are inheritable, thus they can be used for identifying not only escapees but also their offspring in the wild. However, the state of the art of monitoring tools largely varies between species. Variation at a large number of protein loci, mitochondrial sequences, microsatellites and nuclear SNPs has been described for Atlantic salmon, cod, Pacific oyster, European sea bass, and gilthead sea bream. For all these species, genome projects are in progress. DNA chips are also available for studies of gene expression in Atlantic salmon. Genomic studies have also been undertaken for common carp, turbot, halibut, the three mussel species and scallop. However, the number of markers available for these last seven species is still insufficient for complete identification of aquaculture escapees, at least not for all possible circumstances such as when domestic stocks derive from local populations. Their numbers will likely increase as a consequence of genomic developments. To our knowledge, genome projects have not been initiated for the European flat oyster and the European lobster. The forth research priority is thus the identification of genetic markers, together with sampling and statistical methodology, in all species of interest which will allow identification of the farmed stocks.

Note: A particularly useful example of this genetic identification approach was recently seen with Atlantic salmon, where the source of escaped farm fish was identified to the farm from which they escaped (see http://www.imr.no/english/news/2007/farmed_escaped_salmon).

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IV
Management options to reduce genetic impacts of aquaculture activities

The main results obtained in the three expert workshops described in sections I-III, were discussed with important stakeholders during the management workshop in Thessaloniki, Greece.

This chapter reports the results of the discussion focusing on:

1. Developing consensus statements on the genetic impact of farming activities and its implications for aquaculture management, stock conservation and environment safety, and
2. Integrating the scientific basis for the establishment of preventive measures, for important aquaculture species like Atlantic salmon, Atlantic cod, European sea bass, gilthead sea bream, turbot, carp, halibut, scallops, mussels, oysters (Pacific oyster and European flat oyster) and European lobster.

Participants to the Thessaloniki (Greece) workhop, 19th – 22nd April 2007

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Second row (from left): *E. Verspoor, J. Gilbey, L. Holmefjord, H. Nhhala, R. Guyomard, P. A. Prodöhl, K. Joerstad, D. Danancher, I. Olesen, C. Hough*

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Not in the picture: *T.J. Abatzopoulos, E. Babatunde, A. Baxevanis, S. Kalomiris, S. Kampouris, I. Kappas, S. LaPatra, Z. Mamuris, A. Metaxatou, N. Pappas, P. Pavlidou, F. Piferrer, D. Schane, B. Shields, J. Stefanis, C. Vamvakas*

Management options to reduce genetic impacts of aquaculture activities

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Introduction

The potential genetic effects of aquaculture activities have aroused a great deal of concern, and the perceived risks are often associated with interbreeding with natural populations and the adverse effects of ecological interactions. The EU-funded project Genimpact (http://genimpact.imr.no) reviews specific aspects of potential risks and concerns about interbreeding and aquaculture-ecosystem interactions. In workshop 4, the Genimpact scientific team presented results and outputs from previous workshops in various forms, including posters and Power-Point presentations. Discussions followed with aquaculture, breeding, environmental and animal welfare organisations and important points were noted. At the end of the workshop scientists and stakeholders produced consensus statements on the main points discussed, as regards the state of aquaculture of the main species under consideration by Genimpact: Atlantic salmon, Atlantic cod, European sea bass, gilthead sea bream, turbot, common carp, Atlantic halibut, scallops, mussels, oysters (Pacific oyster and European flat oyster) and European lobster.

Aquaculture is currently confronting a set of critical choices that could substantially influence its future development. This is due to the fact that the domestication of aquatic species may benefit from technological advances that were not available at the time of the domestication of terrestrial plants and animals. The number of aquatic animal species entering domestication, already 430, is ten times as great as all domesticated terrestrial animal species, now plateauing at 44. In the past, animal domestication involved two successive stages: first rearing, that is providing shelter, food, care and protected reproduction, and later on selective breeding to rearrange natural genetic variability into phenotypes with better performance. Most aquatic domestication has spread horizontally during the first stage, but only a handful of species have been subjected to improvement programmes according to the protocols of quantitative genetics.

The following are the points of consensus among stakeholders and scientists: 1) on the genetic impact of farming activities and its implications for aquaculture management, stock conservation and environment safety and 2) for the establishment of scientifically based preventive measures for some selected species of importance to European marine aquaculture. The following points should be taken into consideration, not only for these species but for the sustainable development of aquaculture in general.

Main consensus issues

1. Each species should be managed separately

With respect to the level of concern about aquaculture species and their interaction with wild populations, it is not always possible to extrapolate from one species model to others. Every species should be examined independently. No generalised ideas can emerge, since single species/group of species have their own particular life history traits, genetic structure, etc. Much has still to be learnt about the majority of the species under scrutiny, particularly those that are not target species of important commercial capture fisheries, and those from the Mediterranean, because of the high degree of fragmentation of this region. Indeed, basic knowledge is still lacking for most species and appropriate studies are necessary prerequisites for the applied research that would be useful to the industry. One of the most important research priorities would be basic population demographic studies, essential to an understanding of the biology and ecology of the species in the wild and therefore to evaluations of the potential impact of aquaculture. The validity of using Atlantic salmon as a general model for other species, based on the fact that it has been the most studied species so far is questionable, because of the specific biology of salmonids in general that does not resemble that of other groups of species. The potential effect of escapees on wild populations should therefore be assessed on a per species basis.

2. Efforts should be made to avoid escapes

As a sound application of the precautionary principle, it is wise to limit escapes as far as possible, whether or not documented data on their potential impact are already available. Instead of trying to protect wild populations from escapees, the best logical solution would be to try to prevent escapes. This will rely on technical improvements from the industry that have little to do with genetics. There appears to be a growing concern over this problem and a better appreciation of the new challenges that the industry faces as regards more effective confinement techniques. However, realistically, it is almost impossible to completely prevent escapees.

The logical continuation of the debate is therefore: what level of escapes should be regarded as a threat to wild populations? How many individuals can escape before their negative effects on natural stocks are noticeable? This question is another topic that should be dealt on a species basis: for example, Atlantic salmon, mussels and common carp are three extremes managed in completely different ways, which means that the impact of escapees on their respective native populations is unlikely to be the same. The frequency and extent of escapes, the stock structure and the size of the wild populations, as well as the escapees' life stage, are key issues in answering the question posed above. In the case of the common carp, for instance, the definition of what is a wild population is itself a problem, since ferality is commonplace in this species since Roman times. Resilience to the negative impacts of escapes also depends on the relative proportion of gene flow from farmed to wild stocks. Large populations are probably better able to resist the impact of escapes than small ones. Therefore, it is important to stress the fact that wild stocks should be protected to increase their potential to face unwelcome gene flow. Since we still lack a great deal of information on wild populations, such as what is a local population and to what extent these populations have been altered, additional efforts for improving this knowledge are recommended.

As regards the perception of the magnitude of the problem, this may depend on the observer's point of view. Conservationists, environmentalists and non-profit

associations are concerned about aquaculture escapes, but consumers are generally not well informed about the potential environmental or biological impact of aquaculture. Since European citizens are generally concerned about environmental issues, public information about the results of the Genimpact project is regarded as a way of raising public concern about this problem. However, a different point of view stresses that we should not overexpress our concerns to the point of freezing all activities and demonizing the aquaculture industry. According to this view, the environment is continuously changing and local stocks may be more threatened by overfishing and indirect environmental alteration than by aquaculture escapees and, if left in good demographic health, will always try to find ways to adapt locally. The need to set up conservation priorities for wild stocks is emphasised.

3. Measures to identify escapees are needed

In general, traceability of escapees with genetic techniques is considered to be an expensive approach by stakeholders. The aquaculture industry would currently prefer to spend its resources on preventing escapes rather then assessing the impacts of escapes on wild populations. Economic and technical support by governments and fishery authorities is recommended in the area of genetic identification, since it is considered that many farmers cannot afford the costs of traceability studies. The efforts made by the aquaculture authorities and the industry in some countries and cases should nevertheless be acknowledged, such as the search for the identity of escapees as is being done in Norway.

As has frequently been recognised, molecular markers are the tool of choice to identify the origin of escapees. Reliable information on the origin of juveniles and broodstock is highly desirable to assist in traceability. The difficulty of keeping track of broodstock movements for small companies is recognized, since records are rarely kept. Tracing of origin will be even more difficult when different farms keep genetically similar stocks. In the future, broodstock movements and import/exports are actually expected to intensify when breeding programmes in some species become efficient and fish lineages are genetically improved through selection or other methods. A range of situations is expected, again depending on the species under consideration. Growing problems of disease are also expected to occur as broodstock/juvenile movements increase.

4. Input from modelling assessment and risk

Modelling is a tool to aid understanding, identify data deficiencies, define research priorities and provide advice to inform policy and management decisions. The potential for using modelling studies to assess the importance of the frequency and intensity of escapes on the outcome of the interaction of cultured fish with wild populations was recognized. It is important, however, to emphasise that the modelling is an iterative process and an evolving source of information; results of modelling feed into empirical investigations which in turn feed into model development, so that the model is continuously updated and outputs refined, whilst at the same time knowledge gaps are identified and research priorities defined. At the same time, it needs to be emphasised that outputs are not definitive statements; rather they are the implications which emerge from a synthesis of our best understanding. As models are updated with new information and understanding, outputs may change and so the implications of results obtained must be carefully assessed in the context of the completeness of existing understanding.

The assessment on the extent to which outputs from a model can be considered reliable must be made clear when using them as the basis for advice, and otherwise it must be made clear when presenting results that they are preliminary. Failure to do this, where predictions of impact prove inaccurate, has the potential to cause undesirable or unnecessary economic or conservation impacts, and will damage the general credibility of scientific advice used to inform policy and management initiatives. Finally, the feedback showed that when presenting examples of modelling outputs as part of discussions of models and how they work, it is necessary to avoid the impression of bias by showing full range of predicted outcomes under the full range of potential interaction scenarios.

5. Measures to ensure reproductive isolation between wild and domesticated stocks are also needed

Various measures such as hybridization, sterilization and polyploidy can be employed to protect wild populations from escapees. This will in turn depend on a species basis. Much research remains to be done, but sterile fish could form part of the solution. Induction of triploidy has been successfully employed in several fish and bivalves and it is not regarded as a "genetic modification" because it only alters chromosome segregation, without modification of DNA sequences. The main benefit of triploidy is gonadal or gametic sterility achieved with high efficiency, usually between 90 and 100%. Owing to the lack of the counterproductive effects of sexual maturation, better growth, more consistent fillet quality and/or greater final survival are expected in triploids, but they may manifest initial higher mortality, greater costs of fingerling production and sometimes failure to exhibit greater growth. Problems with consumer acceptance are avoidable, because labelling is not required, and polyploidy is commonly found in marketed fruits (up to 8*n* in strawberries) and vegetables. However, the reaction of consumers to sterile/triploid fish will depend on how the information is presented, for example by pointing out the environmental advantages of using sterile individuals for aquaculture production. The most important needs for research are to improve our knowledge of the effects of triploidy on gene regulation and protein function, of the adaptability of triploids to different culture environments and to assess the potential consequences of restocking with triploid fish. However, triploids could indirectly affect wild populations (i.e. ecological impact through predation or competition for resources).

6. New techniques of direct genetic manipulation should be considered

The commercial farming of transgenic fish is generally viewed with widespread scepticism. For example, indefinite moratoria have been enforced on patented GH-transgenic fish. Although it is generally agreed that their consumption would be essentially safe, as they express a protein that is already present in the fish, their possible release into the wild is regarded a serious threat to ecosystem stability and conspecific genetic integrity. It has been concluded that only totally sterile transgenic fish would represent a commercial innovation. Transgenic fish from non-carnivorous species could eventually represent a convenient source of cheap animal protein in countries afflicted by heavy demographic pressure. It is also important to further explore the potential of somatic gene transfer, such as DNA vaccination, which has proved effective against certain fish viruses, though further research is needed to improve administration routes. Finally, transgenesis with RNA interference deserves to be investigated as a possible way of making fish resistant to viral pathogens.

7. Debate on the use of local stocks as broodstocks is ongoing

The main point under consideration, especially for the aquaculture industry, is what constitutes a "farmed" individual. In some cases, fish and shellfish have gone through a process of selection, but in others they are simply captive wild. At the same time, there are also different degrees of domestication even within species, since the impact of domestication on captive individuals is immediate if they survive. Therefore a general answer cannot be given. Initial information from wild local populations should be required in order to determine the phylogeographic structure of wild populations. In cases where there is genetic structure, the use of local stocks for selective breeding is an option worth considering, but the risk of these stocks being uncompetitive in the market should be pointed out. On the other hand, fully domesticated strains could have an e deleterious impact on wild populations, though at the same time the traceability of these selected lineages/broodstocks in the wild will be easier than local stocks. It is quite evident, however, that we still lack a great deal of information on interactions between wild and farmed individuals. The performance of farmed individuals in the wild needs to be examined on a case-by-case basis. Fitness experiments (common garden experiments - similar to those performed on Atlantic salmon) should be carried out on other species and situations.

Stocking (i.e. the deliberate release of hatchery individuals to supplement or enhance wild populations, such as brown trout in most of western Europe and sea bream released into Italian and Greek lagoons) is recognised as a particular and different case. Avoiding stocking is highly recommended but in some cases it may be necessary in order to restore lost populations or to sustain local small-scale fisheries. Wherever possible, the individuals used for restockingshould be produced from captured wild local broodstock

8. Site selection / conservation of wild populations

The siting of aquaculture facilities plays a major role in determining the impact of farmed stocks on wild populations. Conservation measures are also important for protecting wild populations. Priority (as already stated above) should be given to 1) habitat enforcement, 2) sustainment of large wild populations, and 3) control of fishing.

9. Common legislation throughout the EU is needed

We believe that reinforcing voluntary collaboration between the authorities and the industry is much better than imposing protective measures by law. For example, too many constraints on some aspects like broodstock movements between farms are undesirable. Self-regulation of the industry via a voluntary code of practice could be a good way of protecting the wild populations. At the same time it is now realised that there is a growing awareness and willingness by governments as well as the industry to protect wild populations. The need for legislation to implement control and/or compensation measures in some cases should be pointed out. The possibility of establishing a policy of fines or financial compensation for the impact of aquaculture on the wild should be considered.

Differences between countries at this level are recognized; for example, environmental-protective legislation such as that existing in Norway and Scotland has not been developed in other countries. Different management policies in different regions have also been set out. For example, in many countries there is no legal requirement to provide information about aquaculture escapes. In other cases, local governments

have no jurisdiction over aquaculture activities (i.e. movement of stocks, origin of farm broodstocks and others). In some countries only international treaties exist, without further development at national or regional level.

10. Genetics and the aquaculture industry interaction

More interaction between ecologists, quantitative genetics/molecular biologists is recommended because of a lack of exchange of information between different sectors; for example, misunderstandings when specialised terminology is employed. Science must be communicated with clarity, care must be taken that correct terminology is used, and ambiguous scientific terms and ideas should be avoided or defined. Educational programmes aimed at producers are much needed in order to bring information down to the farmers' level, with simple communication tools that can be understood by everyone. At the same time, the participation of scientists in producers' workshops in order to present these issues in an understandable way would be highly desirable.

The information obtained by scientists often does not answer the issues raised by the industry. It is suggested that as far as implications for operating practices are concerned, those actions that will involve the least resistance by producers for the maximum benefit should be applied to the data and recommendations made by scientists.

The application of the genomic revolution is regarded as premature in aquaculture, due to a general lack of understanding and to the technical difficulties involved in applying genomic approaches. However, it is widely felt that the industry will benefit from the genomic revolution as soon as its practical implications become better known. The capacity of the industry to incorporate new techniques to increase productivity and profit should be emphasised. Indeed, we should recognise that the industry has made a major effort to employ scientists and experts in the field of genetics in order to benefit from their know-how and increase production.

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